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1-Methylcyclopropene Inhibits Apple Ripening

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ABSTRACT. **An ethylene action inhibitor, MCP, was applied to preclimacteric and climacteric apple [*Malus sylvestris* L. (Mill.) var. *domestica* Borkh. Mansf.] fruit. Experiments were conducted in North Carolina and Washington State utilizing the following cultivars: Fuji, Gala, Ginger Gold, Jonagold, and Delicious. MCP inhibited loss of fruit firmness and titratable acidity when fruit were held in storage at 0 °C up to 6 months and when fruit were held at 20 to 24 °C for up to 60 days. For all cultivars except 'Fuji', differences in firmness between treated and nontreated fruit exceeded 10 N after 6 months storage. These beneficial effects were seen in both preclimacteric and climacteric fruit. Ethylene production and respiration were reduced substantially by MCP treatment. MCP-treated fruit had soluble solids equal to or greater than those in nontreated fruit. Storage and shelf life were extended for all cultivars tested. Chemical name used: 1-methylcyclopropene (MCP).**

Ethylene has long been known to play a major role in apple ripening. Several compounds have been shown to block the ethylene binding site, causing an inhibition of ethylene effects (Sisler et al., 1990; Sisler, 1991). 2,5-Norbornadiene and diazocyclopentadiene, both inhibitors of ethylene binding, will delay apple softening and ripening (Blankenship and Sisler, 1989, 1993). However, neither of these compounds is commercially acceptable due to toxicity and manufacturing concerns. MCP is an ethylene action inhibitor that has been found to block ethylene responses in plants (Sisler and Blankenship, 1996; Sisler and Serek, 1997). While MCP is a gas, it has been formulated into a powder with the trade name EthylBloc that releases MCP when mixed with a dilute base. The ability of MCP to inhibit ethylene effects has been demonstrated in cut flowers (Serek et al., 1995). The objective of this study was to test MCP as an inhibitor of apple ripening during cold storage and under warm, shelf life conditions, using several apple cultivars grown in two environments, North Carolina and Washington State.

Materials and Methods

FRUIT. Apples were obtained from commercial orchards in Washington State (Wash.) and North Carolina (N.C.). The following cultivars were used: Delicious (Wash. and N.C.), Fuji (Wash.), Ginger Gold (N.C.), Jonagold (N.C.) and Gala (N.C.).

TREATMENT WITH MCP. MCP (as EthylBloc) was obtained from Biotechnologies For Horticulture, Burr Ridge, Ill. Apples were treated in closed containers, either 220 L metal drums fitted with plexiglass lids or 230 L steel chambers with steel lids sealed by a water moat. Fruit were also treated in 4-L glass jars or 22-L plastic containers. In all treatments, MCP was applied as a gas. EthylBloc powder was placed in a beaker on a battery-operated stir plate inside the container. About 5 mL 0.18 $mol \cdot L^{-1}$ KOH in distilled water were added and the lid quickly put in place. Alternatively, EthylBloc powder was placed in a flask sealed with a rubber serum cap and 5 to 10 mL KOH were injected with a syringe through the cap into the flask. The manufacturer provided the weight of EthylBloc powder needed to achieve a specific concentration of MCP gas in a given volume of air. Serial dilutions of concentrated MCP gas were used to achieve the different concentrations of gas. Based on the calculated concentration of MCP, gas was withdrawn from the flask and injected into the sealed container with the apples. Apples were treated for various durations, usually 12 to 16 h, at 20 to 24 °C with a concentration of 0.8 to 1.0 $\mu L \cdot L^{-1}$ MCP as a gas, or as specified.

MEASUREMENTS. Rates of ethylene and carbon dioxide production were measured every other day by sealing 'Delicious' fruit in jars for 1 to 2 h. Concentrations of headspace carbon dioxide and

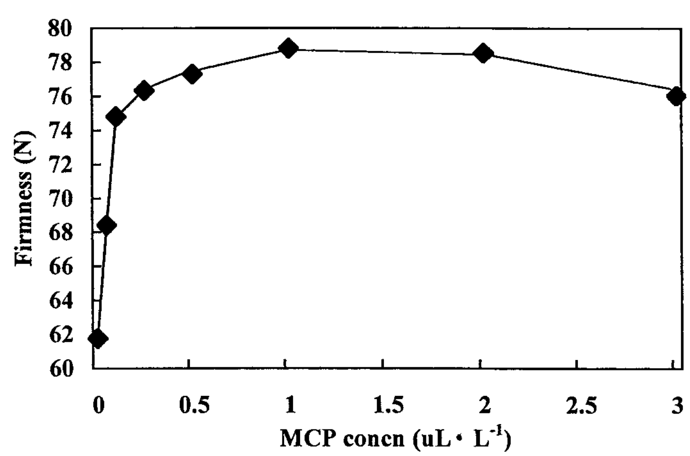

Fig. 1. Effect of MCP concentration on 'Delicious' apple fruit firmness after holding at 20 to 24 °C. Regression equation: $y = 78.5 - 0.44x - 1.19x^2$, $r^2 = 0.93$, $P < 0.01$.

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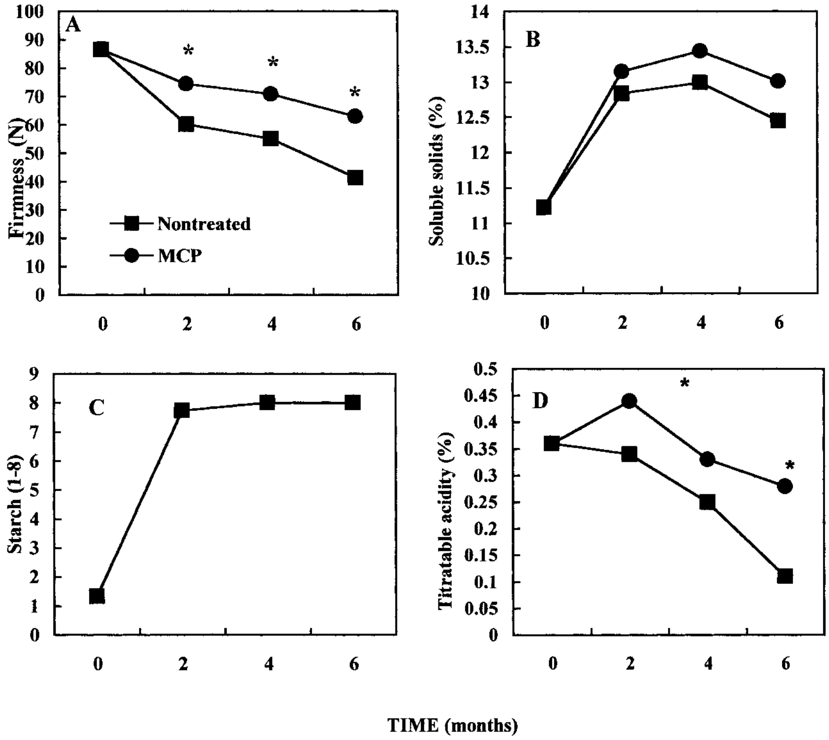


Fig. 2. Effect of MCP on (**A**) firmness , (**B**) soluble solids, (**C**) starch iodine rating, and (**D**) titratable acidity of 'Ginger Gold' apples during air storage at 0 °C. Legend in **A** applies to all figures. In **C** MCP-treated data points hidden under nontreated points. Asterisks indicate significant difference between treatments at that storage time, LSD $P = 0.05$. All regression equations (not shown) significant at $P < 0.01$. Firmness, nontreated (NT) $r^2 = 0.96$, MCP $r^2 = 0.92$; soluble solids, NT $r^2 = 0.92$, MCP $r^2 = 0.89$; starch iodine, NT $r^2 = 0.95$, MCP $r^2 = 0.95$; titratable acidity, NT $r^2 = 0.99$, MCP $r^2 = 0.75$.

ethylene were measured by gas chromatography (HP5890; Hewlett-Packard, Avondale, Pa.) Methods for fruit quality measurements and chromatography done in Wash. have been described previously (Fan et al., 1998). In N.C. experiments, soluble solids were measured on apple juice using a Atago digital refractometer (McCormick Fruit Tech., Yakima, Wash.). Firmness was measured on opposing sides of peeled fruit using a Effegi firmness tester with an 11-mm plunger (McCormick Fruit Tech.). Starch–iodine staining and rating were done using the Cornell chart for comparison (Blanpied and Silsby, 1992). This method uses a 1 to 8 scale, with 1 = all starch and 8 = no starch. Where noted, a 1 to 6 scale was used, with 1 = all starch and 6 = no starch.

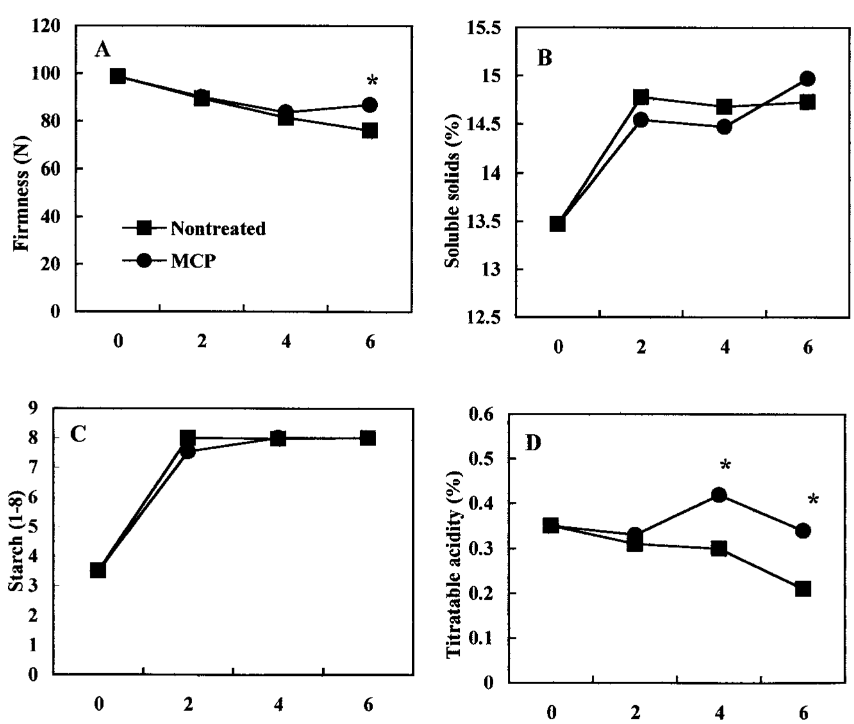
There were 15 apples per sampling time for N.C. experiments. For Wash. experiments 16 or 20 fruit were used (four replicates, four to five apples in each replicate). Storage experiments were at 0 °C for up to 6 months. Shelf life experiments were at 20 or 24 °C for up to 80 d. 'Delicious' used in the concentration curve (Fig. 1) were treated with MCP then held at 20 to 24 °C for ≈2 weeks before firmness measurements were taken. All fruit were held in plastic liners in fiberboard boxes. Statistical analyses, regressions and/or LSD, were performed using SAS procedures (SAS Inst., Inc., 1988).

Results and Discussion

For experiments conducted in both N.C. and Wash., ≈1 $\mu L \cdot L^{-1}$ MCP effectively delayed softening in 'Delicious' when held at 20 to 24 °C (Fig. 1). Exposure time varied from 1 to 12 h depending on diffusion of MCP through the container and/or into the fruit (data not shown).

'Ginger Gold' fruit treated with MCP were always firmer than nontreated fruit (Fig. 2A). 'Gala' showed firmness differences between treated and nontreated fruit only at 6 months (Fig. 3A) 'Jonagold', 'Delicious', and 'Fuji' apples, treated with MCP and stored at 0 °C, were all considerably firmer than nontreated fruit (Figs. 4A, 5A, and 6A). Titratable acidity was greater in MCP-treated fruit for all cultivars during storage (Figs. 2D, 3D, 4D, 5C, and 6C). This would imply that changes in titratable acidity are related to ethylene action. Starch ratings for 'Ginger Gold', 'Gala', and 'Jonagold' were not different between nontreated and treated fruit held at 0 °C (Figs. 2C, 3C, and 4C). Soluble solids were statistically higher in MCP-treated 'Delicious' and 'Fuji' after 6 or 7 months as shown in Figs. 5B and 6B. There were no differences between treatments in soluble solids for 'Ginger Gold', 'Gala', and 'Jonagold' (Figs. 2B, 3B, and 4B). Although

Fig. 3. Effect of MCP on (**A**) firmness, (**B**) soluble solids, (**C**) starch iodine rating, and (**D**) titratable acidity of 'Gala' apples during air storage at 0 °C. Legend in **A** applies to all figures. Asterisks indicate significant difference between treatments at that storage time, LSD $P = 0.05$. All regression equations (not shown) significant at $P < 0.01$, except where noted: Firmness, nontreated (NT) $r^2 = 0.90$, MCP $r^2 = 0.83$; soluble solids, NT $r^2 = 0.59$, $P < 0.05$, MCP $r^2 = 0.55$, $P < 0.05$; starch–iodine, NT $r^2 = 0.90$, MCP $r^2 = 0.93$; titratable acidity, NT $r^2 = 0.90$, MCP $r^2 = 0.18$, $P >$ 0.05.



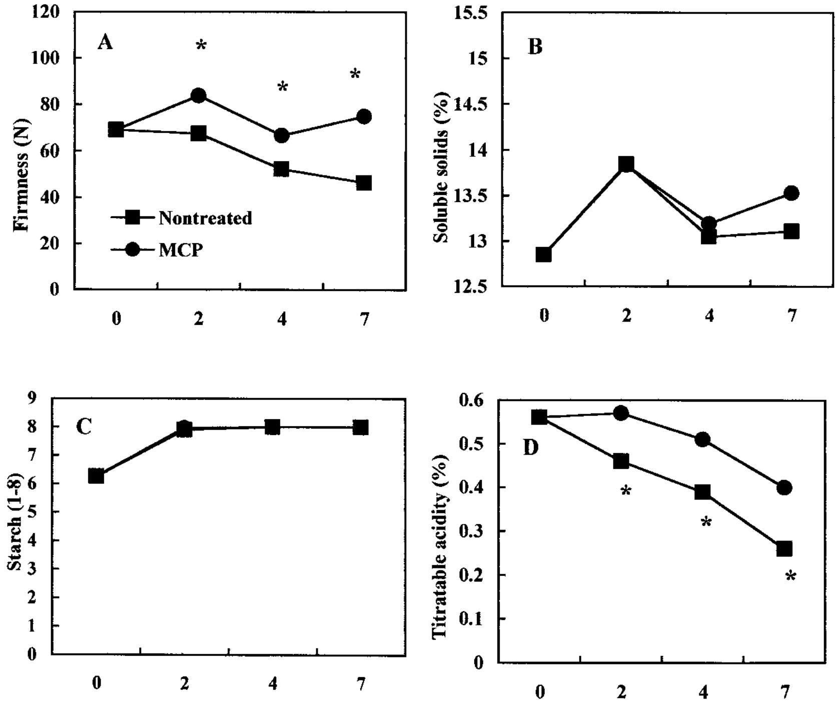


Fig. 4. Effect of MCP on (**A**) firmness, (**B**) soluble solids, (**C**) starch–iodine rating, and (**D**) titratable acidity of 'Jonagold' apples during air storage at 0 °C. Legend in **A** applies to all figures. In **C** MCP-treated data points hidden under nontreated points. Asterisks indicate significant difference between treatments at that storage time, LSD $P = 0.05$. All regression equations (not shown) significant at $P < 0.01$, except where noted: Firmness, nontreated (NT) $r^2 = 0.98$, MCP $r^2 = 0.32$, $P > 0.05$; soluble solids, NT $r^2 = 0.15$, $P > 0.05$, MCP $r^2 = 0.21$, $P > 0.05$; starch iodine, NT $r^2 = 0.79$, MCP $r^2 = 0.78$; titratable acidity, NT $r^2 = 0.99$, MCP $r^2 = 0.99$.

MCP did not always increase soluble solids, it also did not cause a reduction in soluble solids when compared with nontreated fruit. This would indicate that perception of ethylene is not necessarily involved in soluble solids accumulation in apples. Effects of MCP on apples in this study appear similar to those of controlled atmosphere storage (Anderson and Abbott, 1975; Knee, 1976) which suppresses ethylene production and action, resulting in a preservation of firmness and titratable acidity.

Rates of ethylene production and respiration rate were greatly reduced by MCP treatment (Fig. 7). Ethylene production was four fold greater in nontreated fruit than in treated fruit (Fig. 7A). Respiration rate was half as much in MCP-treated fruit as in nontreated fruit between 10 and 25 d storage when control fruit exhibited the respiratory climacteric (Fig. 7B). MCP treatment slowed ripening and reduced ethylene production and respiration in climacteric fruit as well as preclimacteric fruit (Table 1). Ethylene production was actually reduced below harvest values in MCP-treated fruit, while respiration rate was reduced ≈50% at both 3 and 6 months storage. This is in agreement with the finding that diazocyclopentadiene reduces internal ethylene production in fruit at room temperature and during storage (Blankenship and Sisler, 1993).

When 'Jonagold' and 'Delicious' apples were held without refrigeration at 20 to 24 °C, MCP-treated fruit showed little softening after 30 d, while control fruit were extremely soft by 20 d (Figs. 8A and 9A). Similar results were found for 'Ginger Gold' and 'Gala' (data not shown). MCP-treated 'Gala' had a firmness of ≈80 N after 60 days at 24 °C while the controls were at ≈60 N (data not shown). A second treatment with MCP seemed to be beneficial in maintaining firmness and titratable acidity in 'Jonagold' held at warm temperatures for more than 30 d (Fig. 8A and 8D). Titratable acidity was generally higher in MCP-treated 'Jonagold' and Delicious' (Figs. 8D and 9D) and 'Ginger Gold' and 'Gala' (data not shown), when compared with nontreated apples at room temperatures. Soluble solids content and starch rating were not different in 'Jonagold' (Fig. 8B and C), or in 'Ginger Gold' or 'Gala' (data not shown). However, 'Delicious' exhibited higher soluble solids content and slower starch loss in MCP-treated apples (Fig. 9B and C). Studies with the ethylene action inhibitor, 2,5-norbornadiene, on 'Delicious' show little change in the rate of starch loss (Blankenship and Sisler, 1989). However, diazocyclopentadiene, another ethylene action inhibitor similar to MCP, produces an initial retardation of starch loss in 'Delicious'(Blankenship and Sisler, 1993). It is not clear exactly what role ethylene plays in starch and sugar conversions in apples. Titratable acidity was generally higher in MCP-treated fruit, whether treated once or twice (Figs. 8D and 9D). Based on firmness and observed juiciness, some cultivars were acceptable after 60 d at room temperatures, while others were not. By 80 d most fruit were senescent. MCP-treated 'Fuji' apples held 17 d at 20 °C had higher firmness, soluble solids, and titratable acidity than nontreated fruit (data not shown).

Climacteric 'Delicious' apples treated with MCP then held 7 d at 20 °C after 0 °C storage for 3 or 6 months were much firmer and had higher titratable acidity than nontreated

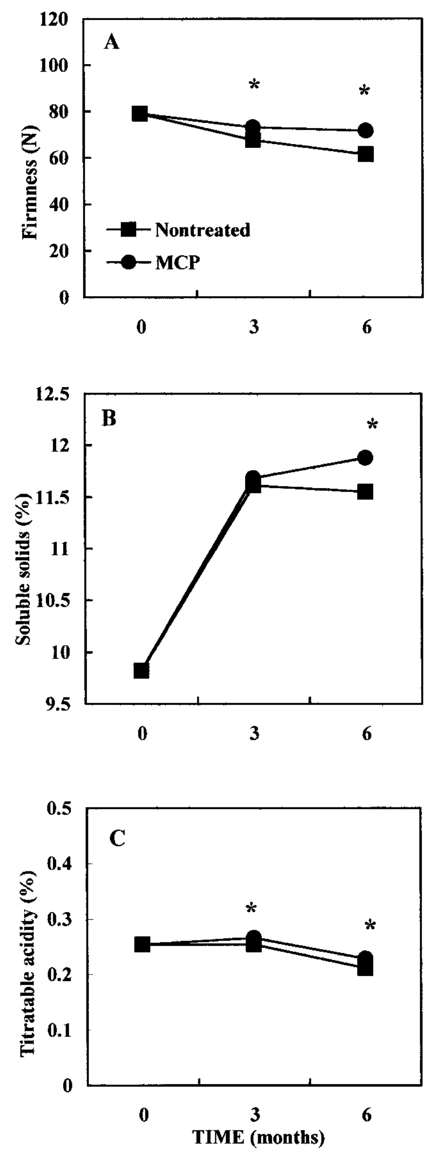


Fig. 5. Effect of MCP on (**A**) firmness, (**B**) soluble solids, and (**C**) titratable acidity of 'Delicious' apples during air storage at 0 °C. Legend in **A** applies to all figures. Asterisks indicate significant difference between treatments at that storage time, LSD $P = 0.05$. All regression equations (not shown) significant at $P < 0.01$: Firmness, nontreated (NT) $r^2 = 0.83$, MCP $r^2 = 0.47$; soluble solids, NT $r^2 = 0.84$, MCP $r^2 = 0.90$; titratable acidity, NT $r^2 = 0.63$, MCP $r^2 = 0.51$.

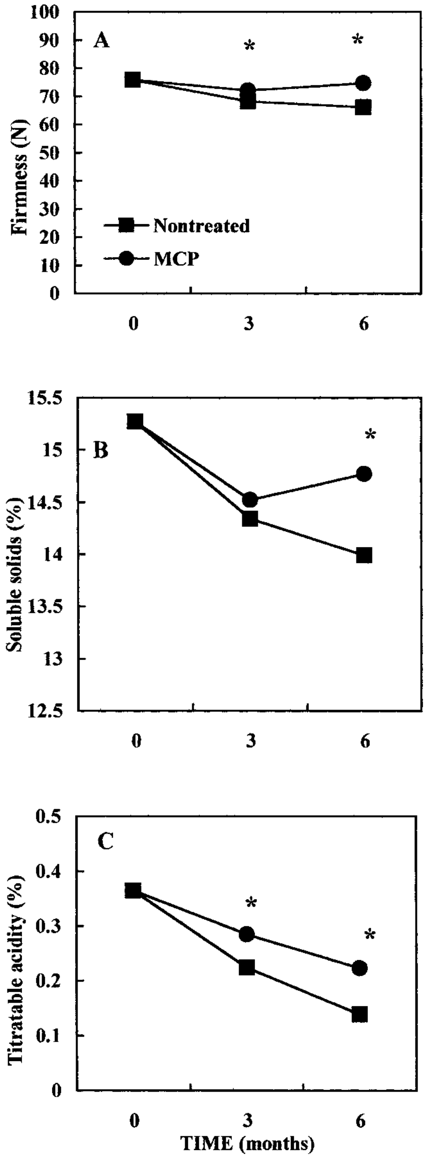


Fig. 6. Effect of MCP on (**A**) firmness, (**B**) soluble solids, and (**C**) titratable acidity of ‘Fuji’ apples during air storage at 0 °C. Legend in **A** applies to all figures. Asterisks indicate significant difference between treatments at that storage time, LSD P = 0.05. Regression equations (not shown) significant at P < 0.01, except where noted: Firmness, nontreated (NT) r^2 = 0.29, MCP r^2 = 0.06, P>0.05; soluble solids, NT r^2 = 0.33, MCP r^2 = 0.19; titratable acidity, NT r^2 = 0.94, MCP r^2 = 0.82.

fruit (Table 1). Soluble solids content was higher in MCP-treated fruit only after 6 months storage and equalized between treatments after holding at room temperature. Fruit softening was reduced substantially following MCP treatment despite the obvious initiation of ripening at harvest. This would indicate that ethylene needs to be present continuously for softening to continue.

In conclusion, apples treated with MCP showed a dramatically reduced rate of fruit softening, maintained higher fruit acidity, and had reduced rates of ethylene production an respiration in comparison with nontreated controls. All apple cultivars had acceptable levels of soluble solids and starch loss after cold storage and holding at room temperature. MCP treatment effectively slowed ripening of all five cultivars tested ranging from softer summer apples, (i.e., ‘Ginger Gold’), to long storage types, (i.e., ‘Fuji’). This compound had a positive effect on both preclimacteric and climacteric fruit. North Carolina and Washington State are very different in climate and fruit production practices, yet MCP effectively slowed apple ripening in fruit produced in both locations.

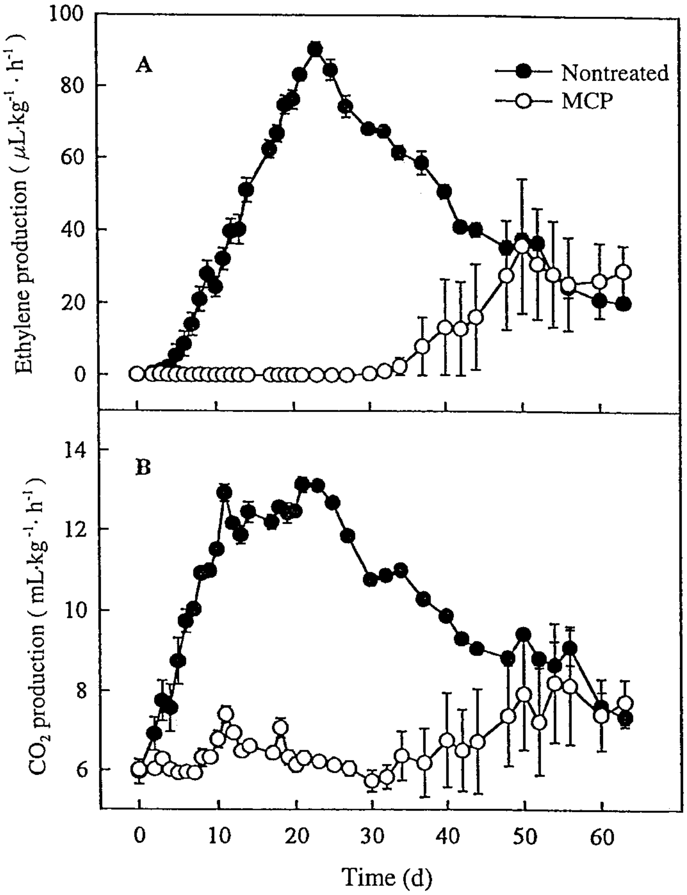


Fig. 7. (**A**) Ethylene and (**B**) carbon dioxide production of ‘Delicious’apple fruit either nontreated or treated with MCP. Legend in A applies to both figures. Vertical bars represent 1 SE.

Table 1. Quality measurements of nontreated or MCP-treated ‘Delicious’ apples after 3 or 6 months storage at 0 °C and 1 or 7 d at 20 °C.

Months at 0 °C	Days at 20 °C	Treatment[z]	Firmness (N)	Soluble solids (%)	Titratable acidity (%)	IEC[y] (μL·L^{-1})	Respiration (mL CO_2/kg/h)
0	0	Harvest	74.0	11.4	0.23	76.48	---
3	1	NT	63.3 a[x]	11.9 a	0.23 a	252.96 a	14.10 a
3	1	MCP	72.0 b	12.1 a	0.24 b	3.50 b	6.07 b
3	7	NT	57.2 a	12.2 a	0.19 a	350.21 a	14.70 a
3	7	MCP	73.2 b	12.2 a	0.23 b	12.25 b	7.24 b
6	1	NT	61.4 a	11.6 a	0.21 a	274.44 a	18.41 a
6	1	MCP	71.7 b	11.9 b	0.23 b	13.32 b	10.12 b
6	7	NT	56.8 a	12.0 a	0.17 a	103.38 a	18.46 a
6	7	MCP	72.4 b	12.4 a	0.22 b	6.89 b	9.20 b

[z]Harvest = initial harvest values, NT = nontreated, and MCP = treated with 1 μL·L^{-1} MCP.
[y]IEC = Internal ethylene concentration.
[x]Mean separation of treatment pairs at each month/day combination for a quality measurement by Fisher’s LSD, P = 0.05.

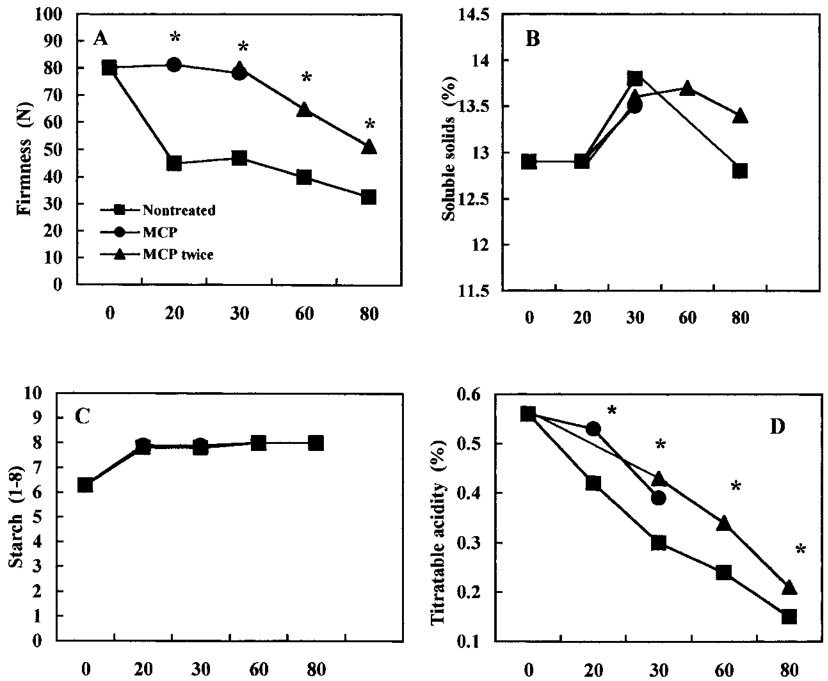


Fig. 8. Effect of MCP on (**A**) firmness, (**B**) soluble solids, (**C**) starch–iodine rating, and (**D**) titratable acidity of 'Jonagold' apples during holding at 24 °C. Some apples were retreated with MCP at 20 d (2× MCP). Legend in **A** applies to all figures. In **C** MCP-treated data points hidden under nontreated points. Asterisks indicate significant difference between treatments at that time, LSD $P = 0.05$. All regression equations (not shown) were significant at $P < 0.01$, except where noted: Firmness, nontreated (NT) $r^2 = 0.89$, MCP $r^2 = 0.28$, $P > 0.05$, 2× MCP $r^2 = 0.96$; soluble solids, NT $r^2 = 0.20$, MCP $r^2 = 0.55$, $P > 0.05$, 2XMCP $r^2 = 0.52$, $P < 0.05$; starch iodine, NT $r^2 = 0.77$, MCP $r^2 = 0.82$, 2× MCP $r^2 = 0.81$; titratable acidity, NT $r^2 = 0.97$, MCP $r^2 = 1.0$, 2× MCP $r^2 = 0.99$.

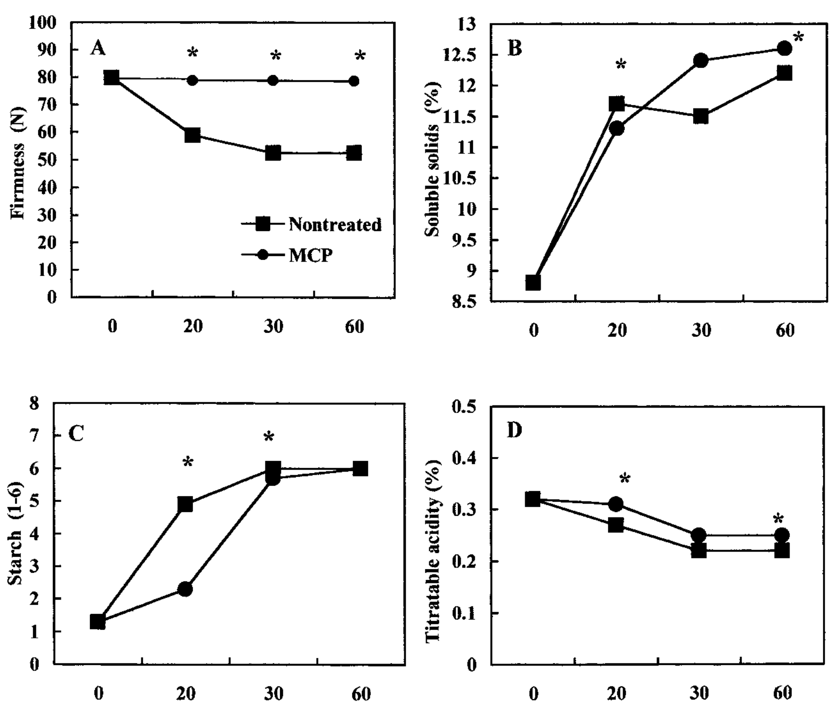


Fig. 9. Effect of MCP on (**A**) firmness, (**B**) soluble solids, (**C**) starch–iodine rating, and (**D**) titratable acidity of 'Delicious' apples during holding at 20 °C. Legend in **A** applies to all figures. Asterisks indicate significant difference between treatments at that time, LSD $P = 0.05$. All regression equations (not shown) significant at $P < 0.01$: Firmness, nontreated (NT) $r^2 = 0.91$, MCP $r^2 = 0.16$; starch–iodine, NT $r^2 = 0.98$, MCP $r^2 = 0.92$; soluble solids, NT $r^2 = 0.81$, MCP $r^2 = 0.92$; titratable acidity, NT $r^2 = 0.70$, MCP $r^2 = 0.62$.

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