ATO

Registration of 1-MCP treatments on Conference pears

Report of experiments storage season 2002-2003

June 2003

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Summary

Experiments on 1-methylcyclopropene (1-MCP, SmartFresh™) were carried out by ATO for AgroFresh Inc. Trials were performed on pear cultivar 'Conference' in the storage season 2002-2003.
For conducting post-harvest treatments of agro-products, ATO has an official recognition to perform efficacy evaluation trials. This research was done following the rules of this recognition.

Treatments with 1-MCP were performed shortly after harvest, during storage or after storage (before shelf-life. Quality of pears was determined after 2, 4, 6 and 8 months of storage. A shelf-life period of 14 days was simulated after each storage period.

A treatment with 25 ppb 1-MCP was not effective. The 50 ppb treatment (before and/or during storage) inhibited firmness loss. Effectiveness decreased with longer storage period. There was no difference between one treatment shortly after harvest and repeated treatments during storage. A negative aspect of 1-MCP treatment is the increase in firmness variability between individual pears.

Treatment with 1-MCP after storage, before shelf-life, was only effective at very high doses (50 ppm).

At a low dose of 1-MCP (25 ppb), the treatment duration is important. There was a clear difference in effectiveness between treatment during 24 h and treatment during 1 or 2 weeks.

Measurements of firmness and/or ethylene production in the period following 1-MCP treatment (after harvest) may be useful for predicting effectiveness of 1-MCP regarding long-term storage.

1 Introduction

Tests with SmartFresh^{tm} (1-MCP, 1-methylcyclopropene) were performed on pear cultivar 'Conference' in the storage season 2002-2003. This pear variety is important in Europe.

Four objectives were formulated before the start of the experiments:
1. What are effects of repeated application of low dose 1-MCP during storage?
2. Is there potential for 1-MCP application at the end of storage, before shelf-life?
3. How does the optimum 1-MCP dose depend on maturity stage at harvest? / Can the optimum 1-MCP dose be predicted at the beginning of the storage period?
4. The effect of 1-MCP if not ventilated out of the treatment room after 24 h.

2 Formal recognition

For conducting post-harvest treatments of agro-products, ATO has an official recognition to perform efficacy evaluation trials. This research was done following the rules of this recognition.

3 Materials and methods

3.1 1-MCP treatment before, during and after storage

Harvest
Pears were harvested on September 4th 2002 (near their optimal harvest date; mean starch index 4.0; advised starch index is 3-5) and September 16th 2002 (mean starch index 4.2). The pears were from an orchard in Randwijk, province Gelderland, The Netherlands (10 km from Wageningen). After harvest, pears were placed in a temperature controlled room at –0.5 °C.

Pre-and post-harvest treatments other than 1-MCP
Pre-harvest treatments within 1 month from harvest:
August 8, 13, 20 and 27 : Eupareen (1.5 kg/ha) and calcium nitrate (4 kg/ha).
No post-harvest treatments other than 1-MCP were done.

Storage issues in commercial storage
Conference is stored in air up to 3 months and in ULO up to 8 months.
Main problems of Conference are development of Brown-Heart and loss of water.

Sorting and randomisation
1 days after harvest, pears were sorted and randomised. Pears that were damaged or very small or very large were removed.

1-MCP treatment before and during storage
Pears were treated with 1-MCP at – 0.5 °C during 24 hours. Treatments were: 0, 25 and 50 ppb starting one day after harvest (September 5th en 17th 2002), using plastic covers. Treatments were repeated during storage. For this repeated treatment, pears were transferred from the storage containers into plastic covers which were conditioned at ±3% O_2.

1-MCP treatment after storage
Part of the pears were treated with 1-MCP only after storage (100, 312, 625 ppb). These treatments were performed in dessicators. A general overview of treatments for objectives 1 and 2 as formulated in 'Introduction' are given in table 1.

Table 1. General overview of treatments for objectives 1 and 2.

Storage	1-MCP dose	application time	Storage duration
ULO	0 ppb (control)		2, 4, 6, 8 months
ULO	25 ppb	each 2 months	2, 4, 6, 8 months
ULO	50 ppb	each 2 months	2, 4, 6, 8 months
ULO	50 ppb	each 4 months	4, 6, 8 months
ULO	100 ppb	after storage	2 months
ULO	312 ppb	after storage	2, 4 months
ULO	625 ppb	after storage	2, 4, 6 months
ULO	1000 ppb	after storage	6 months
Air	0 ppb (control)		2, 4, 6, 8 months
Air	25 ppb	each 2 months	2, 4, 6, 8 months
Air	50 ppb	each 2 months	2, 4, 6, 8 months
Air	100 ppb	after storage	2 months
Air	312 ppb	after storage	2, 4 months
Air	625 ppb	after storage	2, 4 months
Air	1000 ppb	after storage	6 months

To generate 1-MCP levels around the pears, 1-MCP (0.14%) as provided by AgroFresh Inc. was used. For treatment shortly after harvest and during storage, the 1-MCP treatments were performed in plastic

covers (1.38 m^3 volume). In this system airtight plastic is used to cover the fruits which are placed in crates on a pallet. Lime (±10 litre) was placed in each cover to prevent CO_2 accumulation. There was no significant decline in O_2 or rise in CO_2 during treatments. Also in each cover a closed bottle with the desired amount of dissolved 1-MCP was placed. Covers were closed tightly at the top end by a rope and tape. The air inside the covers was circulated by a pump. 1-MCP treatments started by opening the bottles. The flexibility of the cover allowed opening of the bottles from outside the cover. For preparing each solution, a bottle (0.5 l) was filled with the correct amount of powder. The flask was closed with a lid containing a septum. Air was drawn from the closed flask with a syringe. The air volume taken out was equal to the volume of water that was added subsequently. The water (demineralised, ± 20 °C) was added with a syringe. The lid was then wrapped with parafilm. The solution was shaken by hand several times until all the powder had dissolved.
Control pears were placed in a separate room to prevent any possible contact with 1-MCP.

For treatment after storage, 20 L. dessicators were used. The active 1-MCP gas was produced by injecting a surplus of water through a rubber septum in the dessicators into a glass vessel containing SmartFresh™. This solution was stirred briefly.

1-MCP concentration and preparation
The active ingredient of 1-MCP is 0.14%. The free volume inside each pallistore cover was calculated from the total volume inside the cover or dessicator (respectivly 1.38 and 0.02 m^3) minus the volume of pears, crates and lime.
The following solutions were made for a free volume in the pallistore covers of 1.11 m^3:
- 25 ppb 1-MCP: 0.040 g powder + 7 ml water (ratio product:water 160)
- 50 ppb 1-MCP: 0.089 g powder + 14 ml water (ratio product:water 160)
- 100 ppb 1-MCP: 0.187 g powder + 28 ml water (ratio product:water 160)

The following solutions were made for a free volume in the dessicators of 0.0114 m^3 (11.4 L):
- 100 ppb 1-MCP: 0.0018 g powder + 10 ml water
- 312 ppb 1-MCP: 0.0057 g powder + 10 ml water
- 625 ppb 1-MCP: 0.0114 g powder + 10 ml water

Storage conditions
Temperature, relative humidity and ULO conditions were comparable to standard Dutch storage conditions.
After harvest pears were kept under ambient air at –0.5 °C for 22 days. Subsequently part of the pears were stored under ambient air (cooling) and part of the pears under standard CA conditions (ULO, 3% O2 and < 0.7% CO2). ULO conditions started at September 27th (normal harvest) and October 9th (late harvest). Relative humidity during storage was 95-100%. Temperature during the first 43 days after harvest was –0.5 °C, later it was -1 °C (both for air and ULO).

Gas conditions were monitored every hour. All pears were stored in containers. In order to expose pears from different 1-MCP treatments to equal temperature/gas conditions and to reach 2 replicates, pears from the different 1-MCP treatments were stored together in the containers. The number of replicates was 2 (containers).

Quality measurements
Initial quality of pears was measured at the day of harvest. Initial quality of each harvest was measured on 2 samples of 20 pears.
After storage, pears were sampled for quality measurements. A sample consisted of 20 pears. Again 2 samples (replicates) of 20 pears were measured from each treatment. Assessment dates are given in Table 2.

Table 2. Assessment dates for Conference pears directly after storage and after storage plus 7 and plus 14 days shelf-life (both for cooling and for ULO).

Storage duration	Asssessment date
2 months	Nov 4th 2001
2 months + 7 days	Nov 11th 2001
2 months + 14 days	Nov 18th 2001
4 months	Jan 7th 2002
4 months + 7 days	Jan 14th 2002
4 months + 14 days	Jan 21th 2002
6 months	March 5th 2002
6 months + 7 days	March 12th 2002
6 months + 14 days	March 19th 2002
8 months	May 1th 2002
8 months + 7 days	May 8th 2002
8 months + 14 days	May 15th 2002

Directly after storage, pears were measured only on firmness. After a simulated distribution period of 7 days, measurements on more quality aspects were performed. After a simulated distribution period of 14 days, pears were measured only on firmness.
The simulation was performed in a temperature controlled room at 18 °C and 70-85% RH. Measurements were done on firmness (individual pears), colour (individual pears), content of soluble solids (sugars, mixed sample), titratable acidity (mixed sample), and external and internal disorders. Firmness was measured with a fruit texture analyser (Güss, electronic measuring system). Colour was measured visually using a colour chart (Unifruco Research Services LTD/Agricura) with a scale from 0.5 to 5 where 0.5=green and 5=yellow. The content of soluble solids was measured with a digital refracto-meter (ATAGO, PR-1 brix-meter). Titratable acidity was analysed with an automatic titrator using 0.1 M NaOH.

Statistical analysis
Data were analysed statistically without transformation. Different treatments were analysed for significant differences by analysis of variance (ANOVA) with the statistical package Genstat. When significant differences were found, comparisons between pairs of data were made using the least significant differences between means (LSD) at a significance level of 95%. In cases where residuals were not normally distributed and results are clear without using statistics, ANOVA was not performed.

Summary of experimental set-up

Pear variety:	Conference
Harvest dates:	optimal, late
Treatment temperature:	24 h. at –0.5 °C
1-MCP concentrations:	0, 25, and 50 ppb, one day after harvest 25 and 50 ppb after 0, 2, 4 and 6 months 50 ppb after 0 and 4 monhts 100, 325, 625 ppb after storage
Storage gas conditions:	ambient air, CA
Storage temperature:	-0.5 °C during first 42 days, -1 °C during rest of storage period
Sampling dates:	2, 4, 6, 8 months storage
Measurement dates:	0, 7, 14 (days after storage)
Measurements:	firmness, colour, sugars, acidity, external and internal disorders
Pears per measurement:	40 (2 replicates * 20 pears)

3.2 Prediction of optimum 1-MCP dose at the start of the storage period

For this purpose, pears of normal and late harvest date were used. Treatment with 1-MCP took place during 24 h. at –0.5 °C. A part of each harvest was treated with 0 (control), 50, or 100 ppb 1-MCP and ethylene production and firmness was followed during subsequent shelf-life (3, 6, 11, 20, and 31 or 34 days shelf-life). A part of each harvest was treated with 0 (control), 50, or 100 ppb 1-MCP and stored for 2 and 4 months under ULO conditions (Table 3). After storage plus 0, 7 and 14 days shelf-life, quality assessments took place.

Table 3. General overview of treatments for objective 3.

Storage	1-MCP dose	Application time	Storage duration
ULO	0 ppb (control)	after harvest (normal and late harvest)	2, 4 months
ULO	50 ppb	after harvest (normal and late harvest)	2, 4 months
ULO	100 ppb	after harvest (normal and late harvest)	2, 4 months

3.3 The effect of 1-MCP if not ventilated out of the treatment room after 24 h.

To investigate whether it is necessary to ventilate 1-MCP out of the treatment room after 24 h., pears were treated with 1-MCP during 24 h., during 1 week, and during 2 weeks. Treatment was done at 2 °C in closed containers (67 Liter). The 1-MCP was ventilated out of the container after 24 hours, or 1 week or 2 weeks using a pump. All pears stayed inside the containers during 2 weeks. Per treatment, 3 replicates of 15 pears were used. Quality measurements took place directly after these 3 weeks storage + 7 and 14 days shelf-life at 18 °C.

4 Results 1-MCP treatment before and during storage

4.1 Initial quality

The mean firmness of Conference at the start of the experiments was 72.1±0.3N (mean ± standard deviation) for normal harvest and 67.7±1.2N for late harvest. The mean colour was 1.4±0.0 and 1.2±0.0 respectively. The mean content of soluble solids was 10.7±0.4 and 11.4±0.2 °brix respectively. The mean % titratable acid was 0.28±0.01 and 0.16±0.01 respectively.

4.2 2 months storage

Firmness
There was no important firmness loss during 2 months air or ULO storage. Control pears showed severe firmness loss during the subsequent shelf-life (Fig. 1A and 1B). There was no significant effect of 25 ppb 1-MCP treatment. There was a clear effect of 50 ppb 1-MCP treatment on firmness retention however with large variation between individual pears.

Colour
There was no effect 1-MCP treatments on colour (Fig. 1C and 1D).

Soluble solids content
There was no effect of 1-MCP on the soluble solids content (Fig. 1E and 1F).

Titratable acidity
There was no effect of 1-MCP on titratable acidity (Fig. 1G and 1H).

Disorders
There was no effect of 1-MCP on rot development (Fig. 1I and 1J).

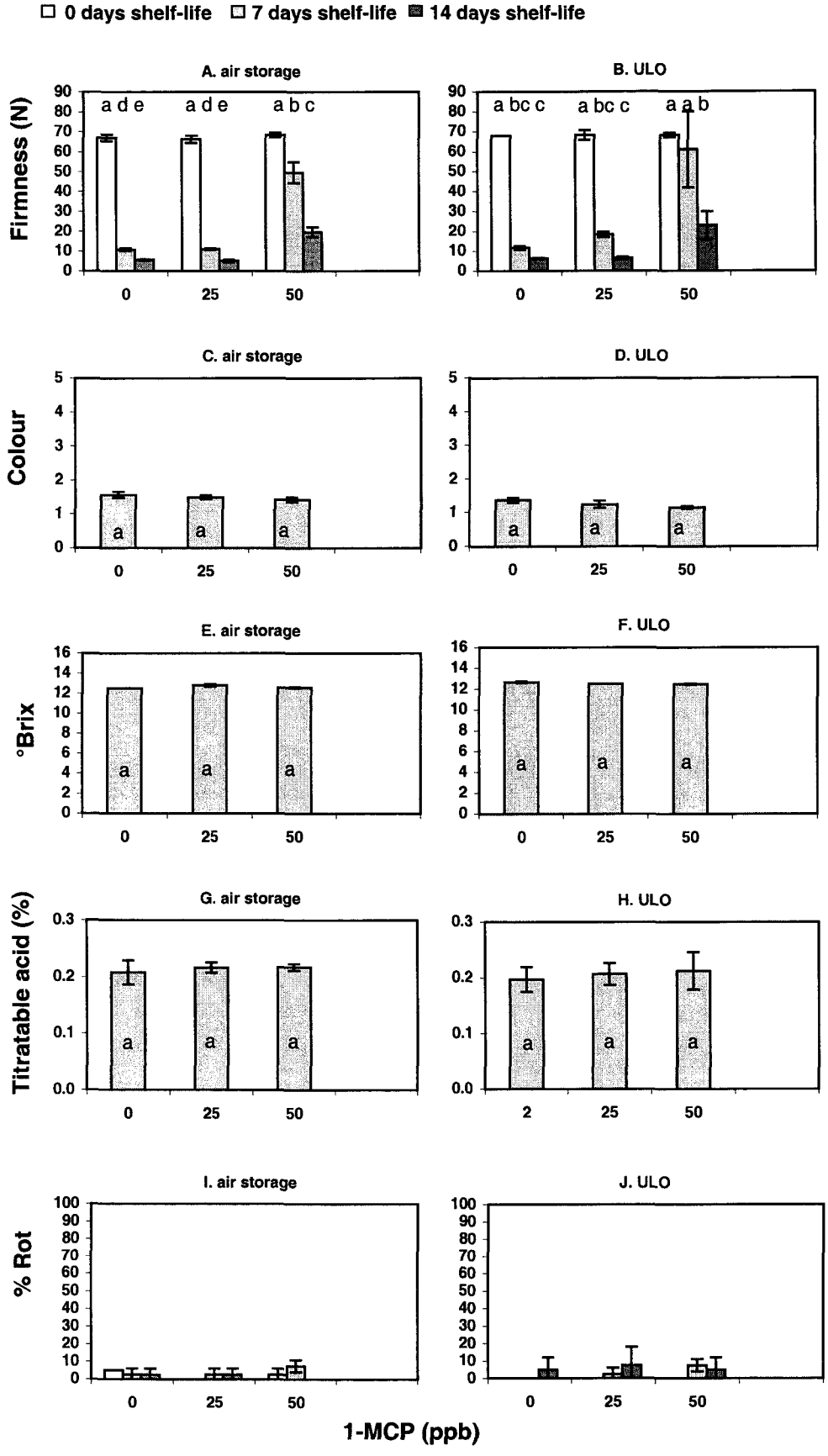

Fig. 1. Quality characteristics of Conference after 2 months of air storage (left) or ULO storage (right). Vertical bars indicate mean ± std of two replicates. Columns within a chart marked with a same letter are not statistically different (P=0.05).

4.3 4 months storage

Firmness
Firmness loss during 4 months air or ULO storage was small and was not affected by 1-MCP. Control pears showed severe firmness loss during subsequent shelf-life (Fig. 2A and 2B). For air storage, there was no effect of 1-MCP. The 50 ppb treatment inhibited firmness loss after ULO storage. There was no difference between 2 treatments ('50' = treatment after harvest and again after 2 months) or 1 treatment ('50 D' = treatment only after harvest). Again there was a large variation between individual pears.

Colour
There was no effect 1-MCP treatments on colour (Fig. 2C and 2D).

Soluble solids content
There was no effect of 1-MCP on titratable acidity (Fig. 2E and 2F).

Titratable acidity
There was no effect of 1-MCP on the soluble solids content (Fig. 2G and 2H).

Disorders
There was no clear effect of 1-MCP on rot development (Fig. 2I and 2J).

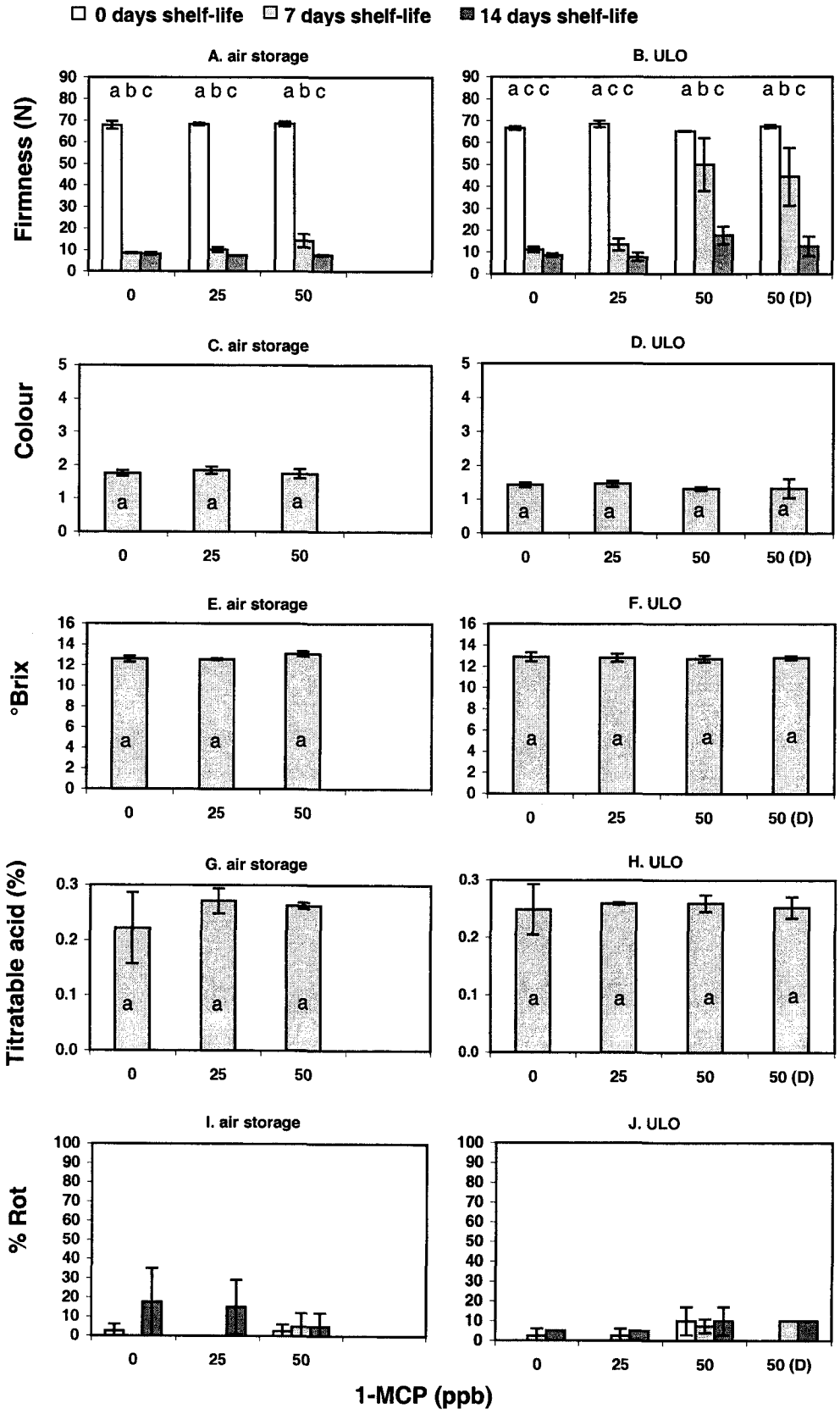

Fig. 2. Quality characteristics of Conference after 4 months of air storage (left) or ULO storage (right). '50' means a treatment after harvest and again after 2 months storage. '50(D)' means a treatment only after harvest. Vertical bars indicate mean ± std of two replicates. Columns within a chart marked with a same letter are not statistically different (P=0.05).

4.4 6 months storage

Firmness
Control pears showed no important firmness loss during 6 months air storage but showed severe firmness loss during shelf-life (Fig. 3A and 3B). For air storage, there was no effect of 1-MCP. The 50 ppb treatment inhibited firmness loss after ULO storage. There was no difference between 3 treatments ('50' = treatment after harvest and again after 2 and 4 months) or 2 treatments ('50 D' = treatment after harvest and again after 4 months). Again there was a large variation between individual pears.

Colour
The only effects on colour were found for air storage: 25 and 50 ppb 1-MCP inhibited yellowing (Fig. 3C).

Soluble solids content
There was no effect of 1-MCP on the soluble solids content (Fig. 3E and 3F).

Titratable acidity
There was no effect of 1-MCP on titratable acidity (Fig. 3G and 3H).

Disorders
There was no clear effect of 1-MCP on rot development (Fig. 3I and 3J).

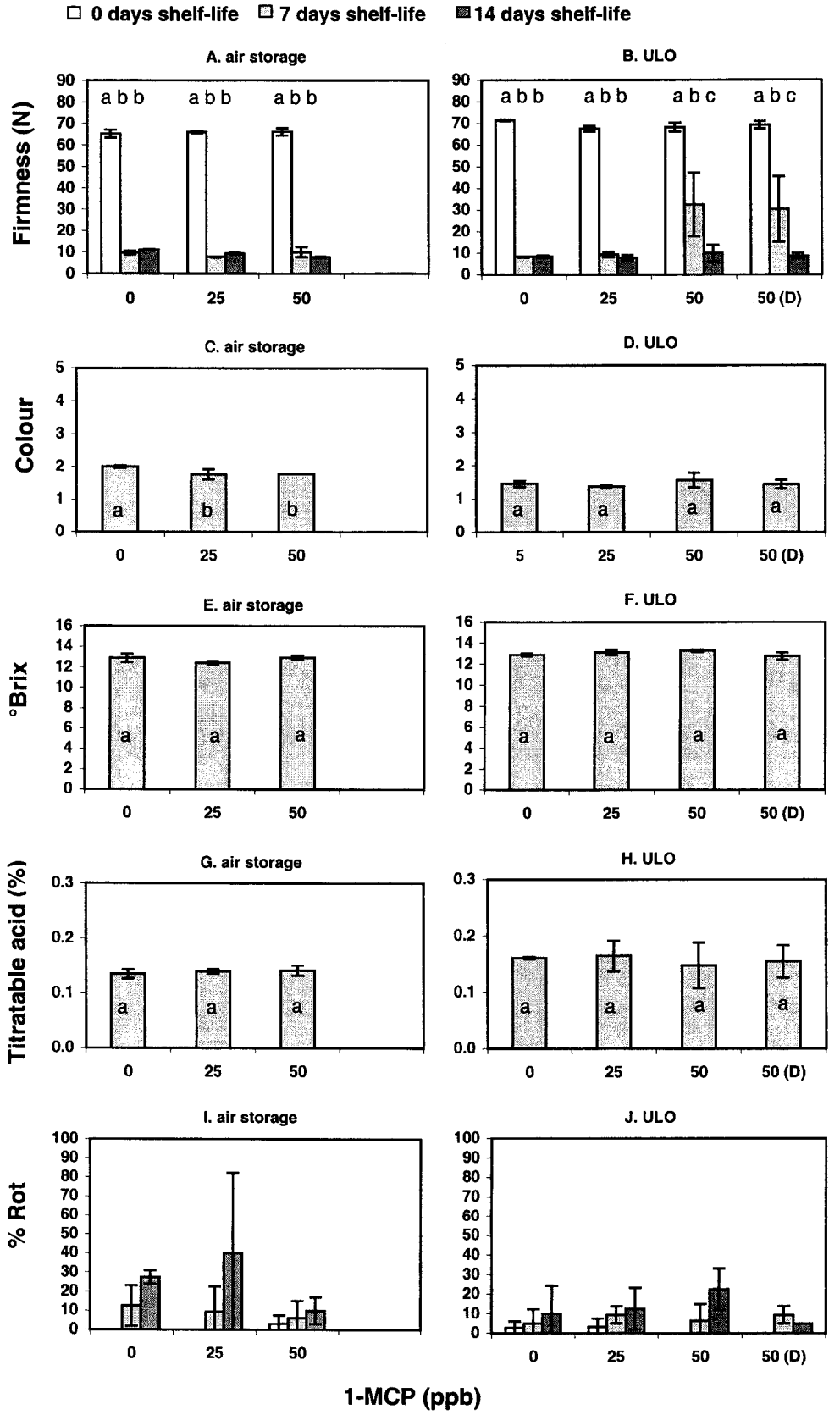

Fig. 3. Quality characteristics of Conference after 6 months of air storage (left) or ULO storage (right). '50' means a treatment after harvest and again after 2 and 4 months storage. '50(D)' means a treatment after harvest and again after 4 months storage. Vertical bars indicate mean ± std of two replicates. Columns within a chart marked with a same letter are not statistically different (P=0.05).

4.5 8 months storage

Firmness
Compared to 2, 4 and 6 months storage, the effect of 1-MCP on firmness loss was small (Fig. 4). A negative aspect of 1-MCP application is the large variation between individual pears (Fig 4B).

Colour
The only positive effect of 1-MCP on colour retention was found air storage: 50 ppb 1-MCP inhibited yellowing (Fig. 4C).

Soluble solids content
There was no effect of 1-MCP on the soluble solids content (Fig. 3E and 3F).

Titratable acidity
There was no effect of 1-MCP on titratable acidity (Fig. 3G and 3H).

Disorders
There was no clear effect of 1-MCP on rot development (Fig. 3I and 3J).

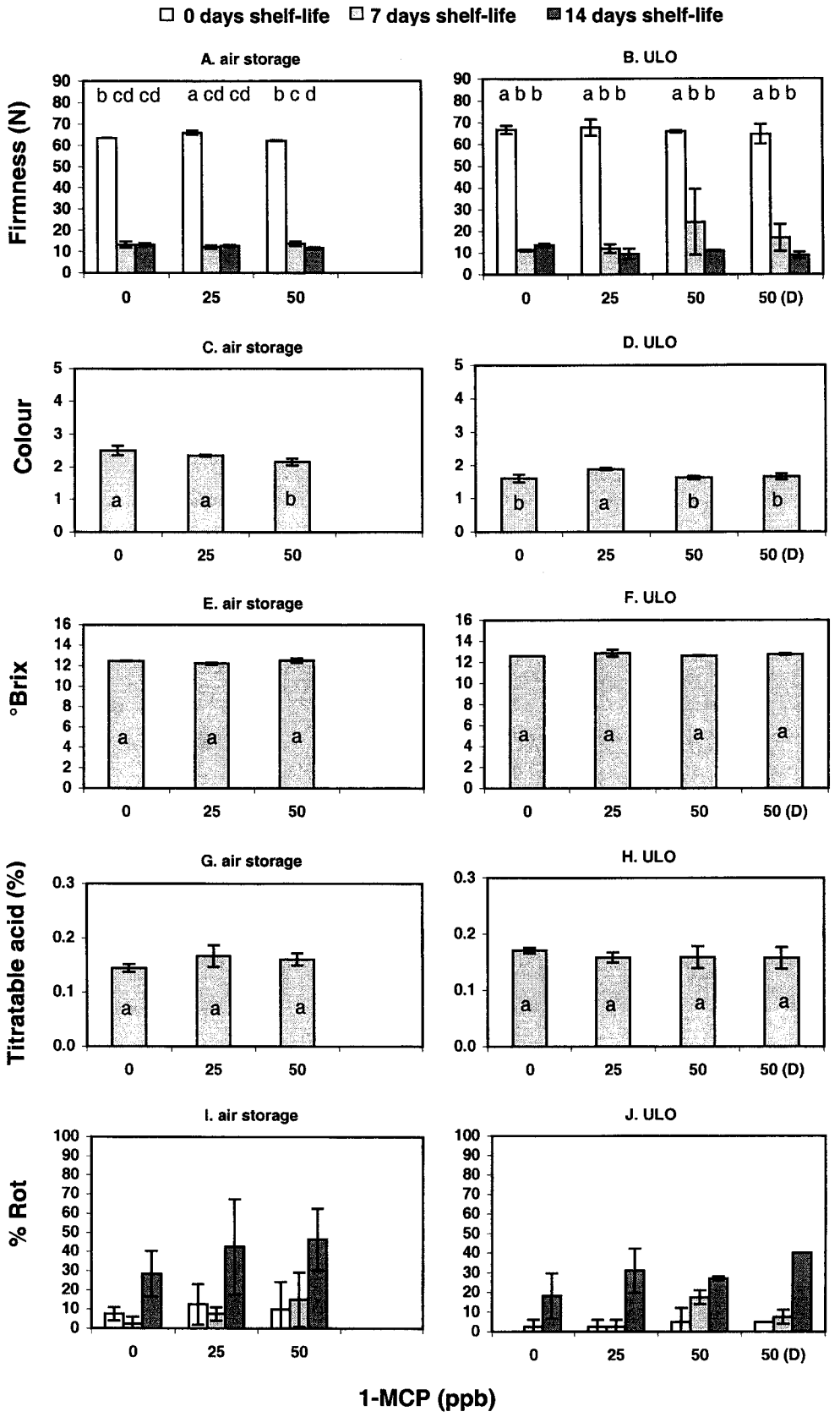

Fig. 4. Quality characteristics of Conference after 8 months of air storage (left) or ULO storage (right). '50' means a treatment after harvest and again after 2, 4 and 6 months storage. '50(D)' means a treatment after harvest and again after 4 months storage. Vertical bars indicate mean ± std of two replicates. Columns within a chart marked with a same letter are not statistically different (P=0.05).

4.6 Brown-Heart

Brown-heart was only scarcely found after 4, 6 and 8 months ULO storage. There was no clear effect of 1-MCP treatment.

5 Results 1-MCP treatment after storage

Treatment with 1-MCP after 2 months storage (applied at –1 °C before shelf-life) showed no effect on firmness retention. (Fig 5A and 5B). Therefore treatment at a higher temperature (18 °C) was tested after 6 months storage. Also this treatment was not effective (Fig. 5C and 5D). A small test was done after 5 months storage with an extreme high 1-MCP dose of 50 ppm (=50000 ppb; not shown in figure). This treatment did inhibited firmness loss: firmness after 7 days shelf-life was 36 and 41 N for 24 hour treatment at –1°C and 18 °C respectively. However, treatment at 1000 ppb and/or a longer exposure time was not effective (Fig. 5E and 5F).

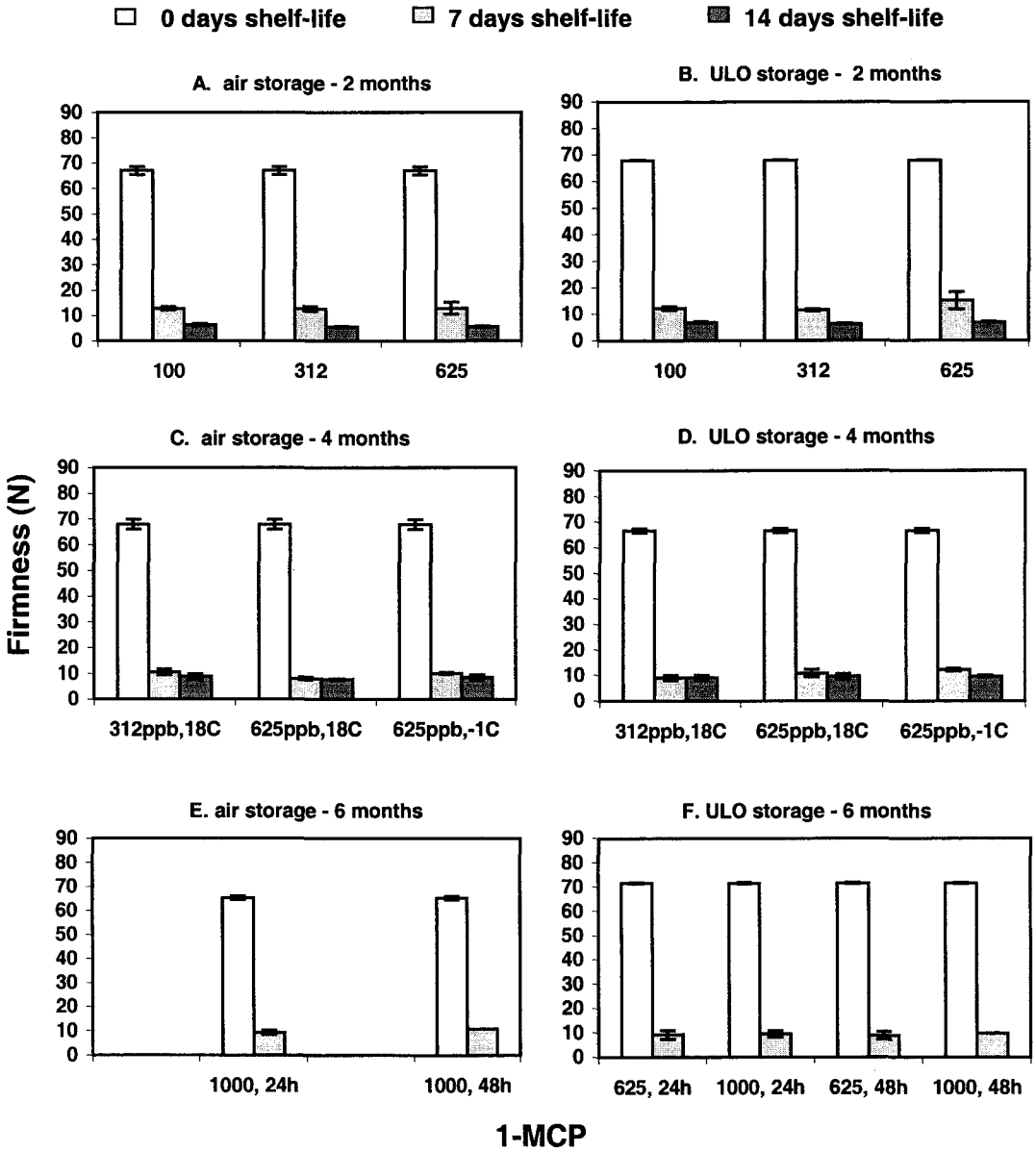

Fig. 5. Firmness of Conference after treatment with 1-MCP after storage (before shelf-life). Vertical bars indicate mean ± std of two replicates.

6 Results, prediction of optimum 1-MCP dose at the start of the storage period

Firmness loss and ethylene production after 1-MCP treatment

Pears, picked at normal and late harvest time, were treated with 0, 50 ppb and 100 ppb 1-MCP immediately after harvest. During shelf life (18°C) ethylene production and firmness were measured. Figure 6 shows the ethylene production (pmol.kg^{-1}.s^{-1}) and firmness (N) of the pears after 3, 6, 11, 20 and 34 (31) days of shelf-life. Without 1-MCP application, pears of normal harvest lost firmness after 6-10 days, pears of late harvest already after 3-6 days. Treatment with 50 ppb 1-MCP partly inhibited firmness loss of normal harvested pears, but not of the late harvested pears. 100 ppb inhibited firmness loss better than 50 ppb and it was more effective with normal harvested than with late harvested pears. In all cases, a clear increase in ethylene production was accompanied with a clear loss of firmness.

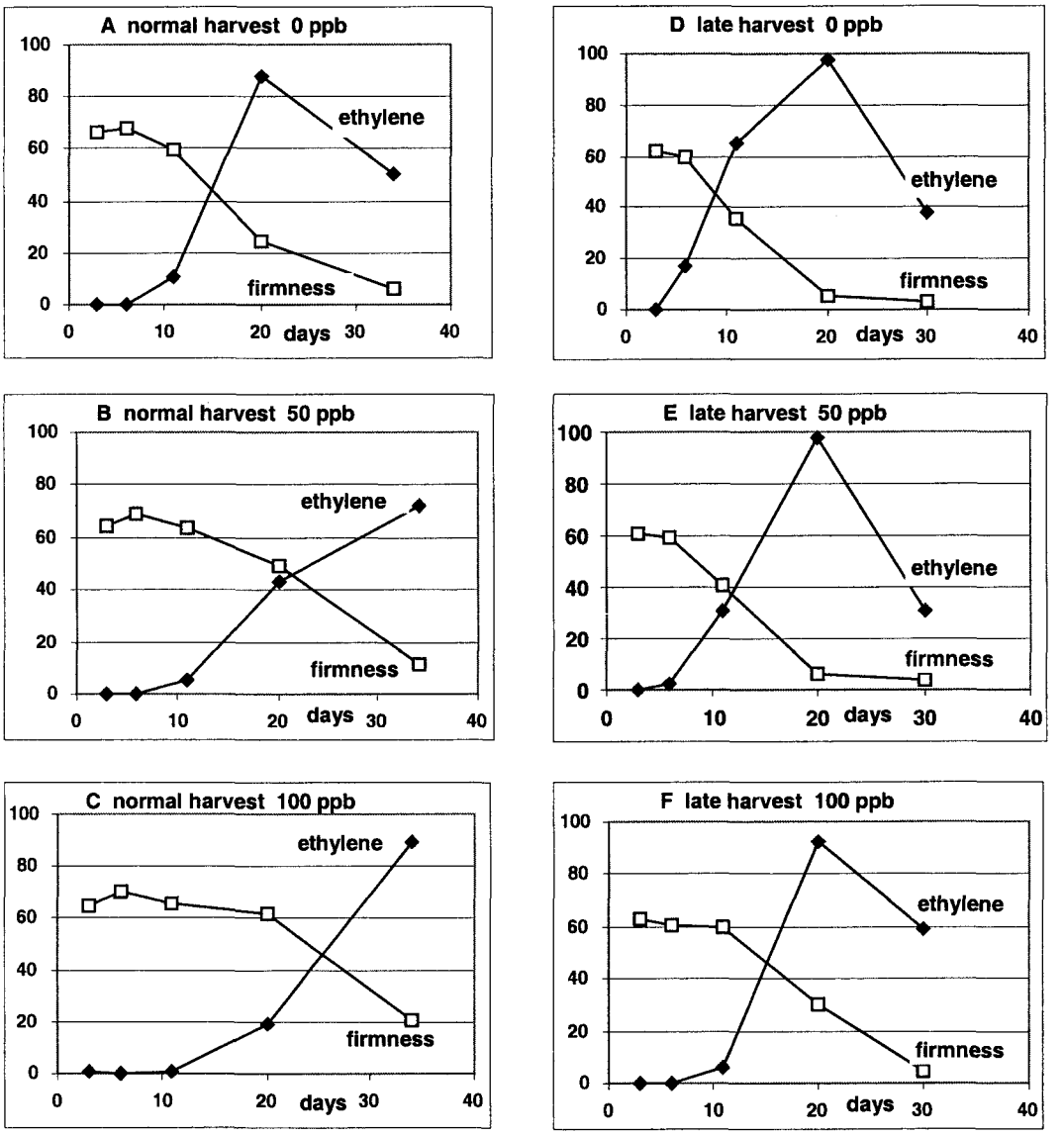

Fig. 6. Relation between ethylene production and firmness of Conference pears (18 °C) after various 1-MCP applications directly after harvest, for normal and late harvest.

Of all the applied 1-MCP treatments, also pears were stored under ULO-conditions for 2 and 4 months. Quality was assessed after storage plus 0, 7 and 14 days of shelf life.

Firmness after storage

For normal harvest, treatment with 50 ppb 1-MCP inhibited firmness after 2 months but only slightly after 4 months of storage (Fig. 7). 100 ppb was effective both after 2 months and 4 months, but effectiveness decreased.

For late harvest, treatment with 50 ppb was not effective. Treatment with 100 ppb was effective after 2 months but hardly showed an effect after 4 months.

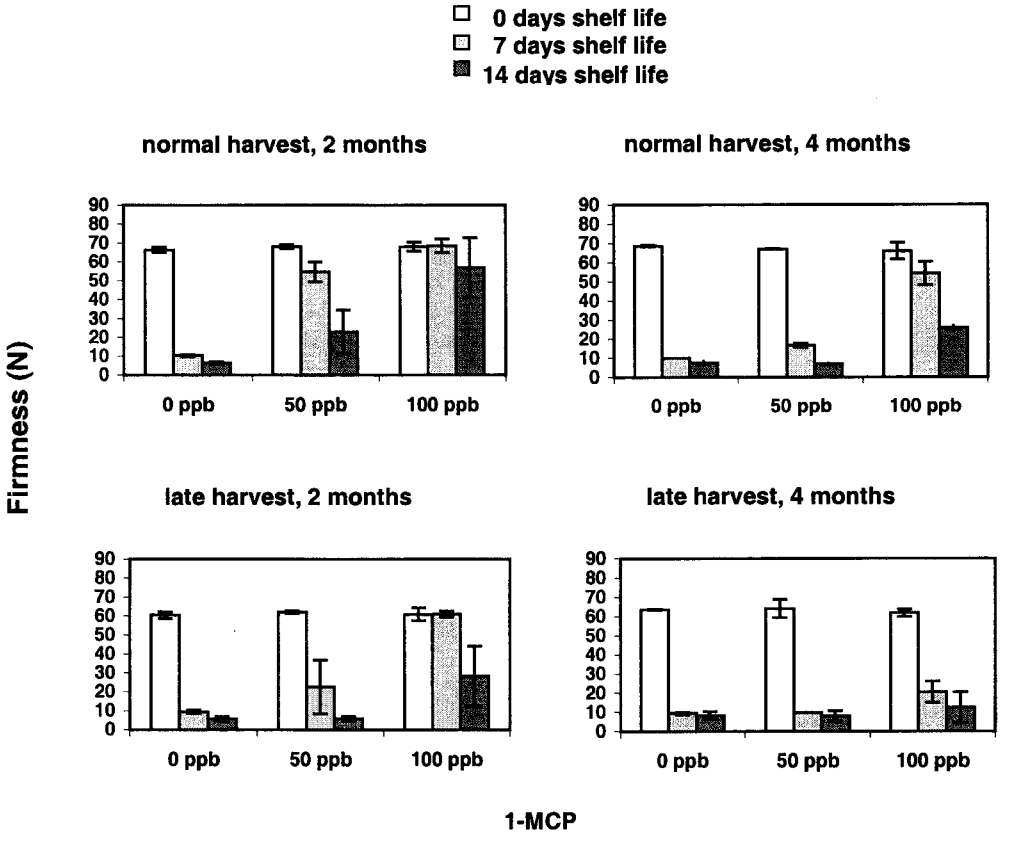

Fig. 7. Firmness of Conference after storage and 0, 7 and 14 days of shelf-life. Vertical bars indicate mean ± std of two replicates.

Colour after storage

After 2 and 4 months of storage plus 7 days shelf life, both the normal as the late harvested pears were still fairly green (fig. 8). 1-MCP had no clear effect on the colour.

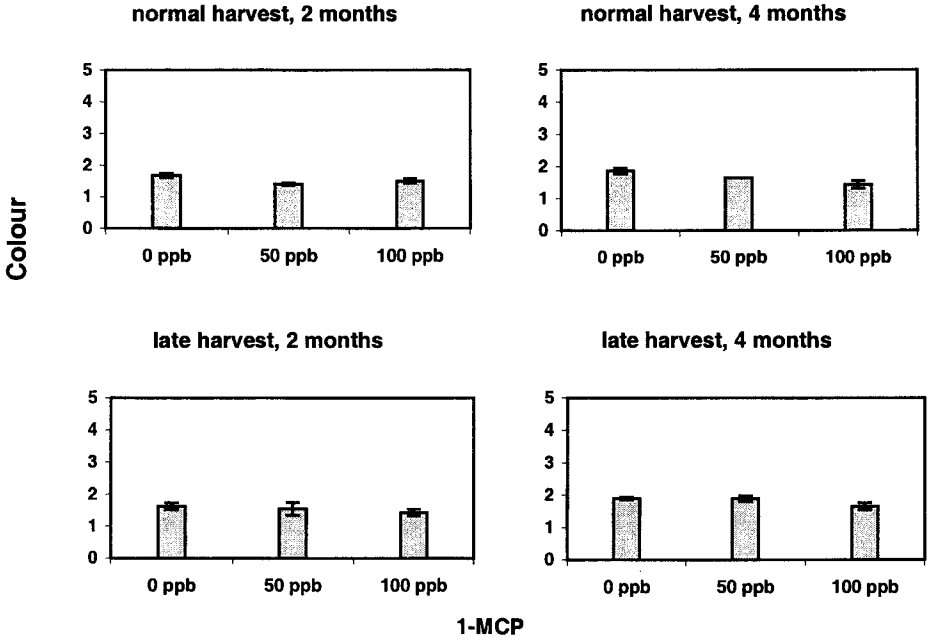

Fig. 8. Colour of Conference after storage and 7 days of shelf-life. Vertical bars indicate mean ± std of two replicates.

Soluble solids content after storage

There was no important effect of 1-MCP on the soluble solids content (fig. 9).

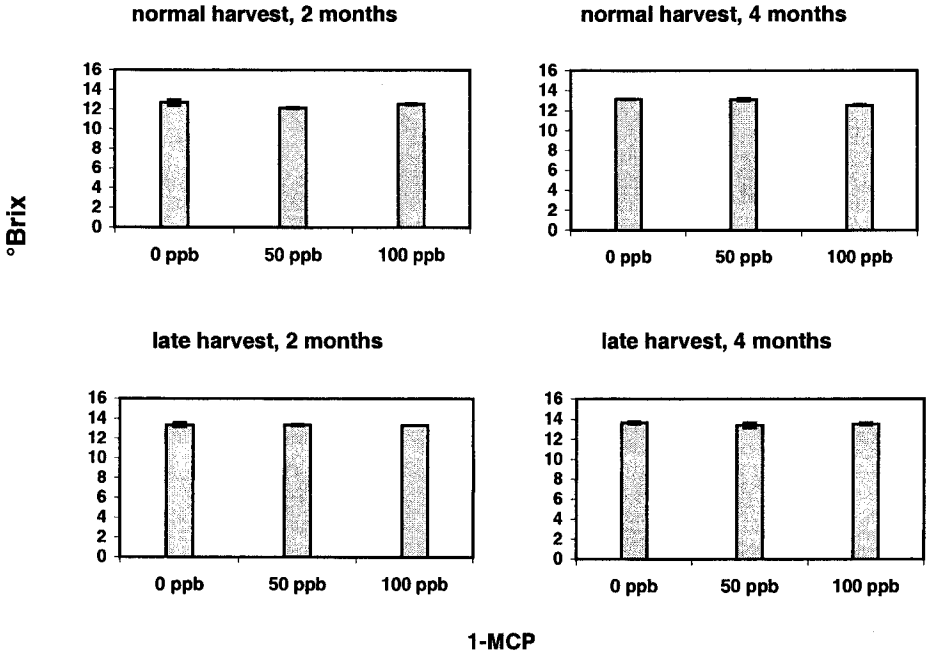

Fig. 9. Soluble solids content of Conference after storage and 7 days of shelf-life. Vertical bars indicate mean ± std of two replicates.

Titratable acidity after storage

There was no efect of 1-MCP on the titratable acids (fig 10).

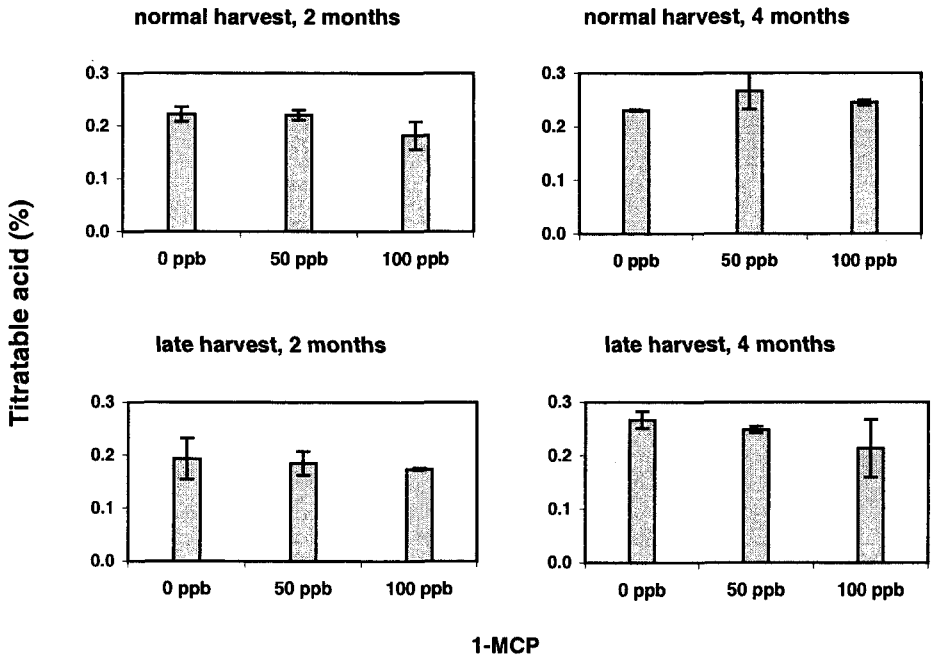

Fig. 10. Soluble solids content of Conference after storage and 7 days of shelf-life. Vertical bars indicate mean ± std of two replicates.

Disorders

Brown-Heart and rot were scarcely found, without effect of 1-MCP.

Conclusion

Measurements of firmness and/or ethylene production in the period following SmartFresh treatment (after harvest) may be useful for predicting effectiveness of 1-MCP regarding long-term storage: for instance 50 ppb was effective for normal harvest but not for late harvest.

8 Results, the effect of 1-MCP if not ventilated out of the treatment room after 24 h.

Pears were treated with 1-MCP during 24 h., during 1 week, and during 2 weeks. As all pears were still firm after 2 weeks in the containers at 2 °C, firmness was not determined at 0 days shelf-life, but after 7 and 14 days shelf-life at 18 °C. Pears that were treated for 24 hours with 25 ppb 1-MCP lost firmness during 7 days shelf-life but were still firmer than the untreated pears (Fig. 11). After 14 days shelf-life the pears treated for 24 hours were soft. Pears treated for 1 or 2 weeks were still firm after 7 days shelf life and had slightly lost firmness after 14 days shelf-life. There were no clear differences in colour, soluble solids content or acidity between the different 1-MCP treatments.

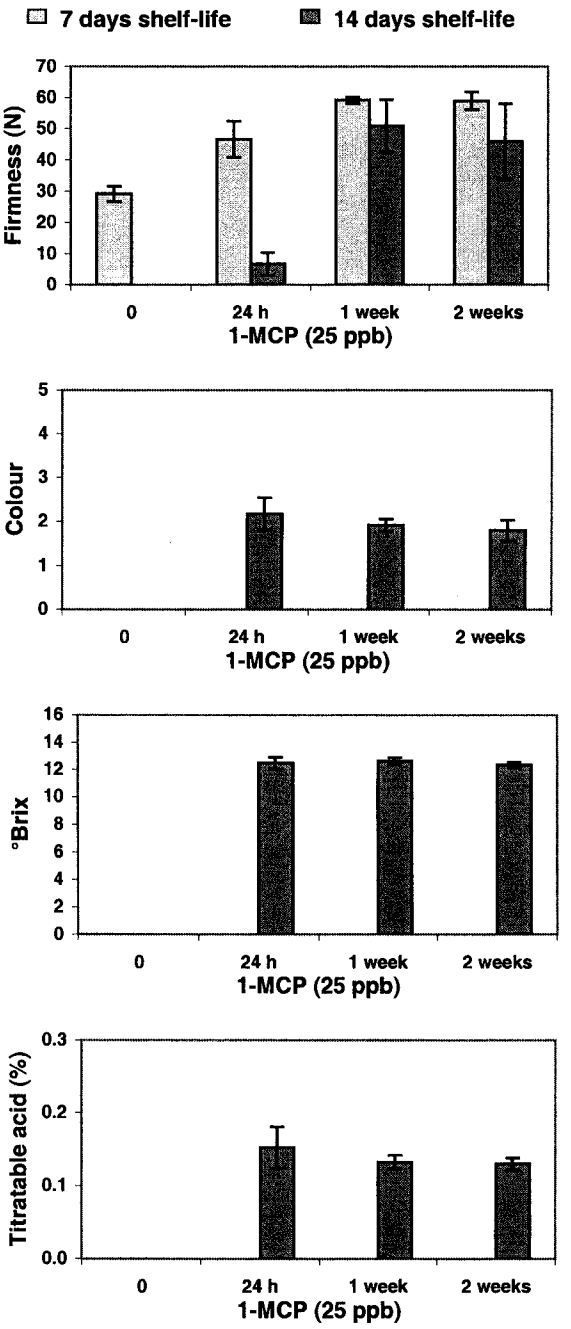

Fig. 11. Quality characteristics of Conference after different exposure duration to 1-MCP (25 ppb) after 7 and 14 days shelf-life. Vertical bars indicate mean ± std of two replicates.

9 Conclusions

The concentration and time of 1-MCP application are very important as pears must have an acceptable firmness at the time of consumption.
The 25 ppb treatment was not effective. The 50 ppb treatment (before and/or during storage) inhibited firmness loss. Effectiveness decreased with longer storage period. There was no difference between one treatment shortly after harvest and repeated treatments during storage. A negative aspect of 1-MCP treatment is the increase in firmness variability between individual pears.

Treatment with 1-MCP after storage, before shelf-life, was only effective at very high doses (50000 ppb).

At a low dose of 1-MCP (25 ppb), the treatment duration is important. There was a clear difference in effectiveness between treatment during 24 h and treatment during 1 or 2 weeks.

Measurements of firmness and/or ethylene production in the period following 1-MCP treatment (after harvest) may be useful for predicting effectiveness of 1-MCP regarding long-term storage. Late harvested pears require a higher 1-MCP dose to get the same effect as pears of normal harvest date. By following ethylene production and firmness loss directly after treatment, it appeared that the 50 ppb 1-MCP treatment was effective for normal harvest, but not for late harvest. For normal harvest, the 100 ppb treatment was slightly more effective than 50 ppb. For late harvest, the 100 ppb treatment was effective, however less compared to normal harvest.
These results were confirmed by measurements after storage: 50 ppb treatment was effective for normal but not for late harvest; the 100 ppb treatment was more effective than 50 ppb treatment.