ATO

Registration of 1-MCP treatments on Conference pears

Report of experiments storage season 2001-2002

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Confidential

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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 2001-2002.
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.

Pears were harvested near the optimum picking date. Treatments with 1-MCP (10, 50, 100 ppb) were performed shortly after harvest. Subsequently pears were stored in normal air (cooling) or in ULO conditions (low O_2, high CO_2). Quality of pears was determined after 2, 4, 6 and 8 months of storage. A shelf-life period of 7 days was simulated after each storage. Also, a treatment with 10 ppb 1-MCP was repeated every 2 months during storage.

There were no clear effects of 10 ppb 1-MCP (one treatment or repeated each 2 months) on quality characteristics. The 50 and 100 ppb 1-MCP treatments had positive effects on firmness retention during shelf-life. If softening is required for consuming, the 1-MCP effect may sometimes be to strong.
The 1-MCP effect depends also on storage duration. Therefore, decision shortly after harvest about the best 1-MCP concentration is difficult.
In some cases, 1-MCP inhibited yellowing and led to lower acidity.
Pears in this experiment were sensitive to the disorder Brown-Heart as was shown by the high % of control pears with this problem. In general, 1-MCP treated pears showed more Brown-Heart.

1 Introduction

1-Methylcyclopropene (1-MCP) is an effective inhibitor of ethylene responses. To test the efficacy of 1-MCP on pears, experiments were performed on pear cultivar 'Conference' in the storage season 2001-2002. This variety is important in Europe.
Three objectives were formulated before the start of the experiments:

- To reveal the efficacy of 1-MCP treatments on various quality aspects of CA (ULO) stored pears. In other words: does 1-MCP improve CA storage?
- To reveal the efficacy of 1-MCP treatments on various quality aspects of pears in comparison to CA (ULO) storage. In other words: can 1-MCP be used to replace CA under certain conditions?
- Does 1-MCP improve the shelf-life of CA(ULO)-stored pears?

Pears were harvested near the optimum picking date. The treatments of 1-MCP were performed shortly after harvest. Subsequently pears were stored in normal air (only cooling) or in ULO conditions (low O_2, high CO_2). One treatment (10 ppb) was repeated every 2 months. Quality of pears was determined after 2, 4, 6 and 8 months of storage and after a simulated shelf-life period of 7 days. The experiments were done following the rules of formal recognition.

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

Harvest
On September 18th 2001 pears were harvested. Pears were harvested near their optimal harvest date (commercial advice; advised starch index is 3-5). Harvests were within the window of commercial harvests. 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 21th : TMTD 3.0 kg/ha
August 29th : TMTD 3.0 kg/ha; Naa 100 ml/ha;
September 6th : Eurpareen 1.5 kg/ha; Mantrac 0.75 L/ha ; Naa 100 ml/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
Pears were treated during 24 hours with 1-MCP at – 0.5 °C. Treatments were: 10, 50 and 100 ppb starting one day after harvest (September 19th 2001). Also control pears (0 ppm 1-MCP) were included in the experiments. A treatment with 10 ppb 1-MCP was repeated each 2 months during storage (September 19th 2001, January 28th 2002, March 27th 2002). For this repeated treatment, pears were taken from the ULO containers and treatments were done under normal air conditions.
To generate 1-MCP levels around the pears, 1-MCP (0.14%) as provided by AgroFresh Inc. was used. The 1-MCP treatments were performed in plastic covers (1.16 m^3 free volume, 140-160 kg pears). 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. This lime does not absorb 1-MCP, which was tested. 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.
Control pears were placed in a separate room to prevent any possible contact with 1-MCP.

1-MCP concentration and preparation
The active ingredient of 1-MCP is 0.14%. The free volume inside each pallistore cover was calculated to be 1.16 m^3. The following solutions were made:
- 10 ppb 1-MCP: 0.019 g powder + 3 ml water (ratio product:water 160)
- 50 ppb 1-MCP: 0.093 g powder + 15 ml water (ratio product:water 160)
- 100 ppb 1-MCP: 0.186 g powder + 30 ml water (ratio product:water 160)

For preparing each solution, a bottle (0.1 or 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.

Storage conditions
Temperature, relative humidity and ULO conditions were comparable to standard Dutch storage conditions.
During and after the 1-MCP treatment all pears were kept under ambient air at –0.5 °C for 21 days.
Subsequently part of the pears were stored under ambient air (cooling) and part of the pears under standard CA conditions (ULO). ULO conditions started at October 10th. Relative humidity during storage was 95-100%. Temperature from harvest to October 31th was –0.5 °C, later it was -1 °C (both for air and ULO).

ULO conditions	: September 19th – October 10th	: normal air
	October 10th – November 14th	: 3% O_2 and <0.7% CO_2
	November 14th – end of storage	: 2.5% O_2 and <0.7% CO_2

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 within a few days after 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 1.

Table 1. Assessment dates for Conference pears directly after storage and after storage plus 7 days shelf-life (both for cooling and for ULO).

Storage duration	Asssessment date
2 months	Nov 15th 2001
2 months + 7 days	Nov 22th 2001
4 months	Jan 15th 2002
4 months + 7 days	Jan 22th 2002
6 months	March 15th 2002
6 months + 7 days	March 22th 2002
8 months	May 15th 2002
8 months + 7 days	May 22th 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.
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. Disorders included flesh and core browning (Brown-Heart) and rot.

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%.

Summary of experimental set-up

Pear variety:	Conference
Harvest dates:	optimal
Treatment temperature:	24 h. at –0.5 °C
1-MCP concentrations:	0, 10, 50, and 100 ppb, one day after harvest 10 ppb every 2 months
Storage gas conditions:	ambient air, CA
Storage temperature:	-0.5 °C, -1 °C after October 31th
Sampling dates:	2, 4, 6, 8 months storage
Measurement dates:	0, 7 (days after storage)
Measurements:	firmness, colour, sugars, acidity, external and internal disorders
Pears per measurement:	40 (2 replicates * 20 pears)

4 Results

4.1 Initial quality

The mean firmness of Conference at the start of the experiments was 62.3±1.3N (mean ± standard deviation). The mean colour was 1.5±0.5. The mean content of soluble solids was 12.0±0.3 °brix respectively.

4.2 Conference, 2 months storage

Firmness
There was no significant firmness loss during 2 months air or ULO storage. Control pears showed severe firmness loss during the subsequent 7 days shelf-life (Fig. 1A and 1B). Pears from the highest 1-MCP treatment (100 ppb) did not loose firmness during the shelf-life period. The effect of 10 ppb 1-MCP was small. The effect of 50 ppb was moderate, probably leading to pears with good firmness for consumption, both for air and ULO storage.
Firmness at harvest was 62.3±1.3 N, the firmness after 2 months storage without 1-MCP was 60.7±2.5 (air) and 62.6±0.3 N (ULO).

Colour
There was no effect of 10 ppb 1-MCP treatment on colour (Fig. 1C and 1D) while the 50 and 100 ppb treatment inhibited yellowing. This was the case both after air storage and after ULO.

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

Titratable acidity
For ULO storage, both 50 and 100 ppb 1-MCP caused a lower % of titratable acids compared to control pears (Fig 1H).

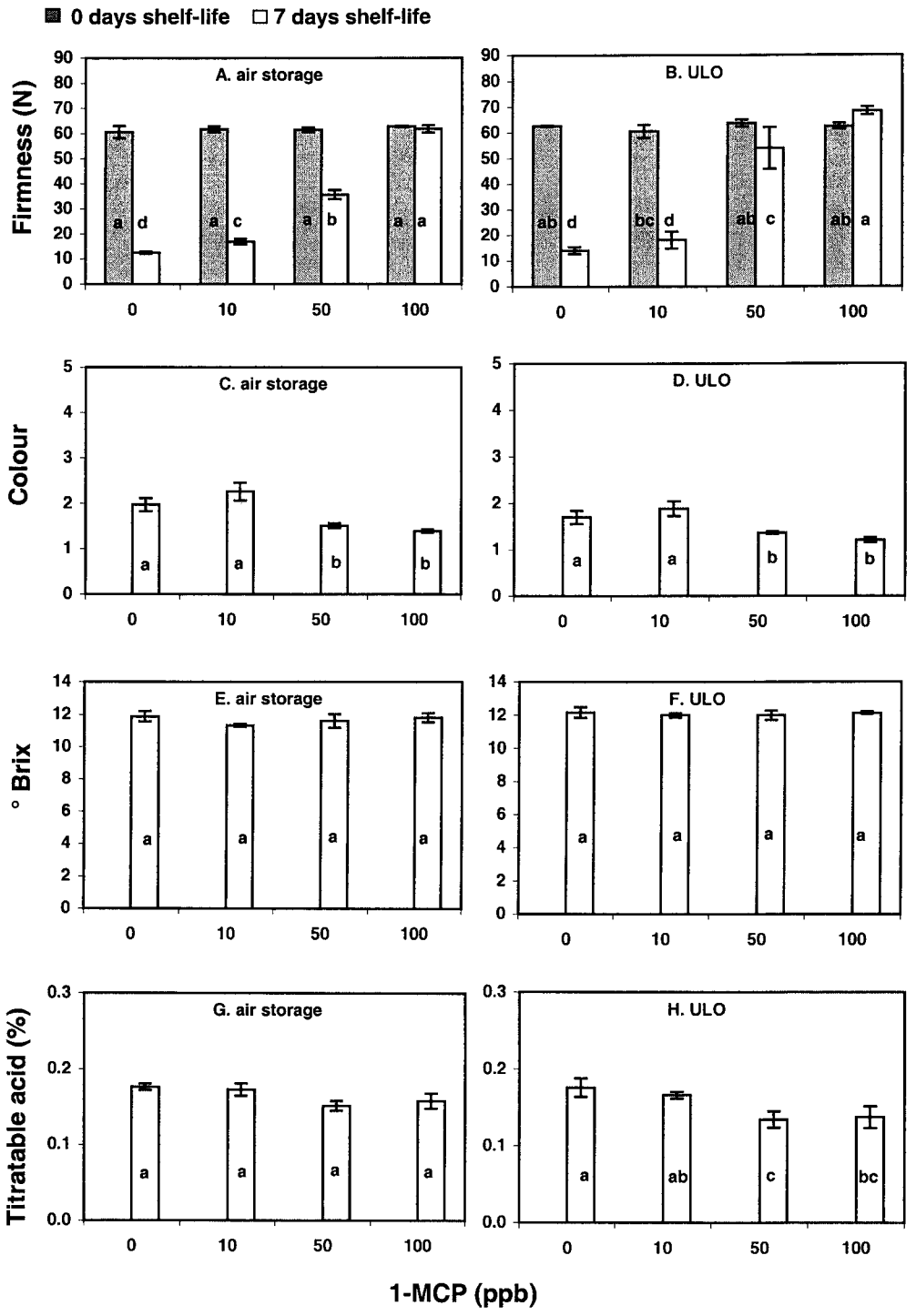

Fig. 1. Quality characteristics of Conference after 2 months of air storage (left) or ULO storage (right). Vertical bars indicate mean ± std of two replicates. Columns within a chart marked with a same letter are not statistically different (P=0.05).

4.3 Conference, 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). There was no effect of one or repeated treatments with 10 ppb 1-MCP. The 50 and 100 ppb treatments inhibited firmness loss, especially after ULO storage.

Firmness at harvest was 62.3±1.3 N, the firmness after 4 months storage without 1-MCP was 57.8±1.3 (air) and 60.7±0.1 N (ULO).

Colour

The effect on colour was similar to 2 months storage: There was no effect of 10 ppb 1-MCP (Fig. 2C and 2D) while 50 and 100 ppb 1-MCP inhibited yellowing.

Soluble solids content

There was no effect of 1-MCP on the soluble solids content (Fig. 2E and 2F).

Titratable acidity

There was no effect of 1-MCP on titratable acids (Fig. 2G and 2H).

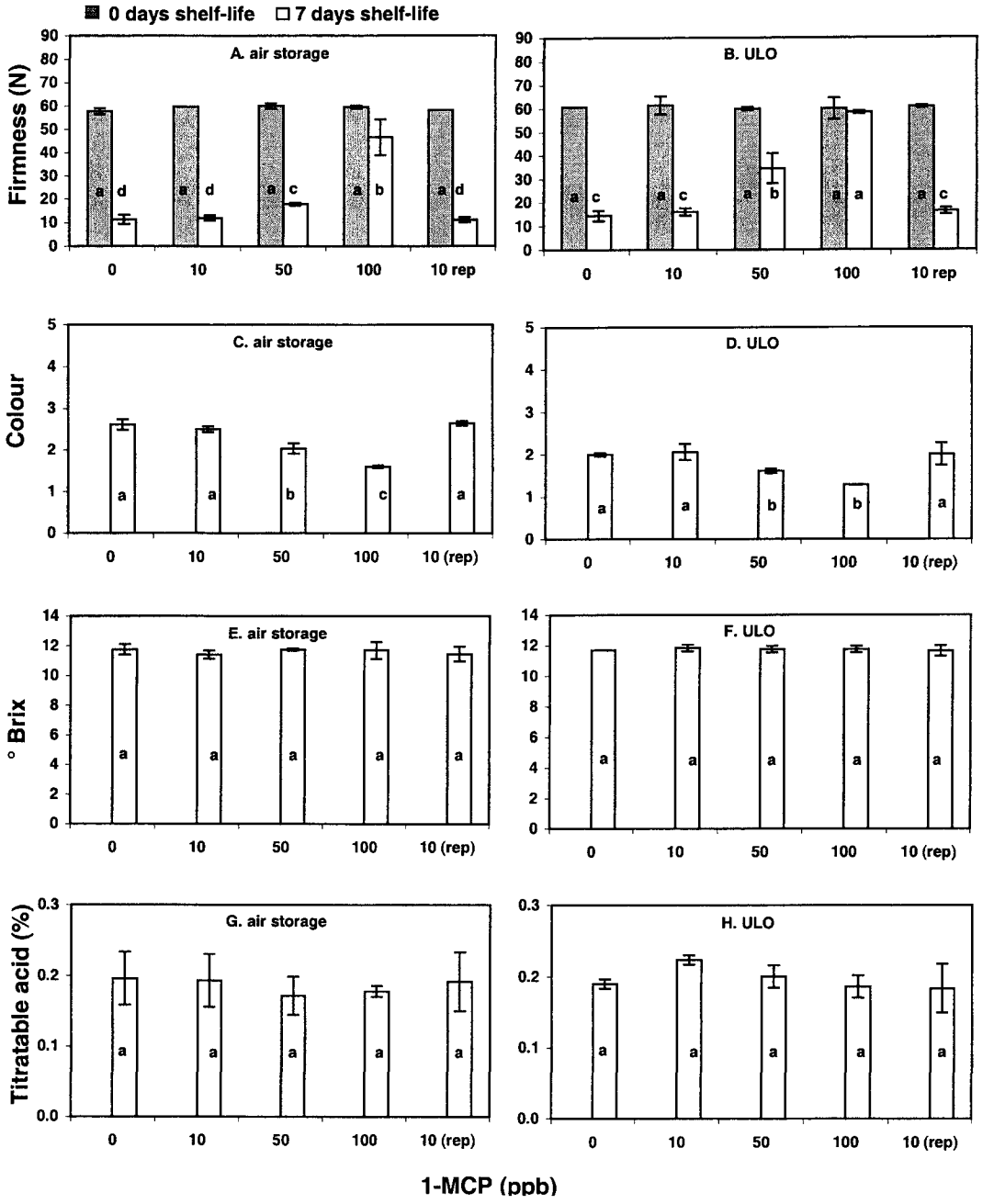

Fig. 2. Quality characteristics of Conference after 4 months of air storage (left) or ULO storage (right). 10 rep means a repeated treatment with 10 ppb 1-MCP. Vertical bars indicate mean ± std of two replicates. Columns within a chart marked with a same letter are not statistically different (P=0.05).

4.4 Conference, 6 months storage

Firmness

Control pears showed firmness loss during 6 months air storage. This was largely prevented by 100 ppb 1-MCP. Firmness loss during ULO storage was small, without clear effects of 1-MCP.
Control pears showed severe firmness loss during shelf-life (Fig. 3A and 3B). There was no effect of (repeated) treatment with 10 ppb 1-MCP. The 50 and 100 ppb treatments inhibited firmness loss. However, inhibition was less strong compared to 4 months storage. The standard deviation at 100 ppb 1-MCP after shelf-life was relatively high.

Firmness at harvest was 62.3±1.3 N.
The firmness after 6 months air storage was 53.7±2.3 N, 54.1±2.0N, 55.8±1.7N, 57.8±2.0N and 57.1±1.1 N for 0, 10, 50, 100 and 10 (rep) ppb 1-MCP respectively.
The firmness after 6 months ULO storage was 58.0±0.2 N, 57.9±1.2N, 57.2±2.9N, 56.6±0.9N and 56.0±1.4 N for 0, 10, 50, 100 and 10 (rep) ppb 1-MCP respectively.

Colour

The only effects on colour were found for air storage: 50 and 100 ppb 1-MCP inhibited yellowing (Fig. 3C and 3D).

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 acids (Fig. 3G and 3H).

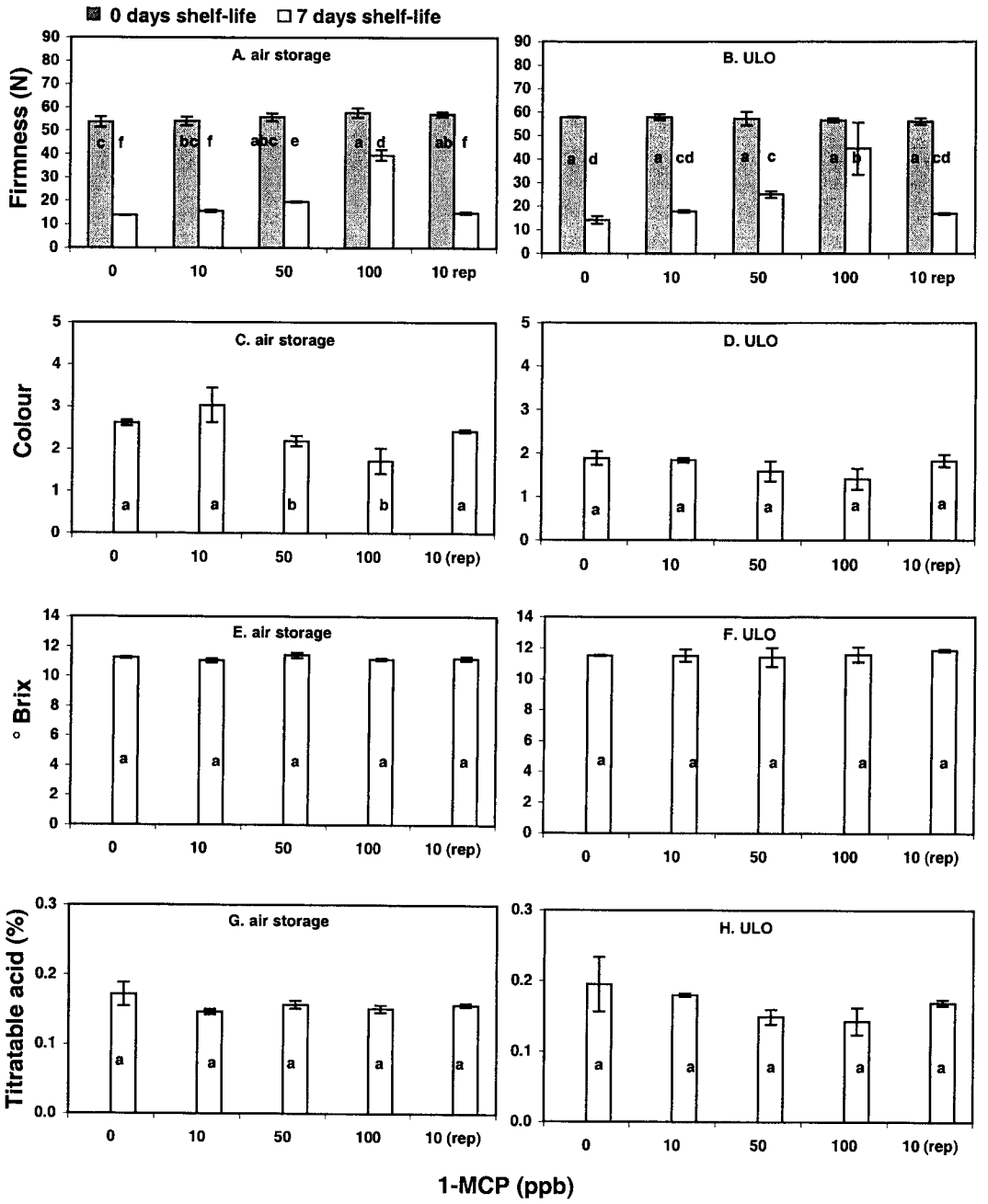

Fig. 3. Quality characteristics of Conference after 6 months of air storage (left) or ULO storage (right). 10 rep means a repeated treatment with 10 ppb 1-MCP. Vertical bars indicate mean ± std of two replicates. Columns within a chart marked with a same letter are not statistically different (P=0.05).

4.5 Conference, 8 months storage

Firmness

Control pears showed approximately 6 N firmness loss during 8 months air storage and 3 N during ULO. There was no effect of 1-MCP (Fig. 4A and 4B). Firmness loss during shelf-life was only inhibited by 100 ppb 1-MCP treatment, but this effect was small. Similar to 6 months storage, the standard deviation at this treatment was relatively high.

Firmness at harvest was 62.3±1.3 N.
The firmness after 8 months air storage was 56.5±0.0 N, 55.7±0,.6N, 57.0±0.3N, 57.1±0.4N and 56.6±0.4 N for 0, 10, 50, 100 and 10 (rep) ppb 1-MCP respectively.
The firmness after 8 months ULO storage was 59.6±1.4 N, 57.8±1.8N, 59.3±0.2N, 58.2±1.6N and 57.4±1.7 N for 0, 10, 50, 100 and 10 (rep) ppb 1-MCP respectively.

Colour

Similar to 6 months storage, the only effects on colour were found for air storage: 50 and 100 ppb 1-MCP inhibited yellowing (Fig. 4C and 4D)

Soluble solids content

Effect on soluble solids content was only found for ULO: 100 ppb 1-MCP led to slightly higher soluble solids content compared to control pears (Fig. 4E and 4F).

Titratable acidity

Effect on titratable acids was only found for ULO: 100 ppb 1-MCP treatment led to lower % titratable acids compared to control pears (Fig. 4G and 4H).

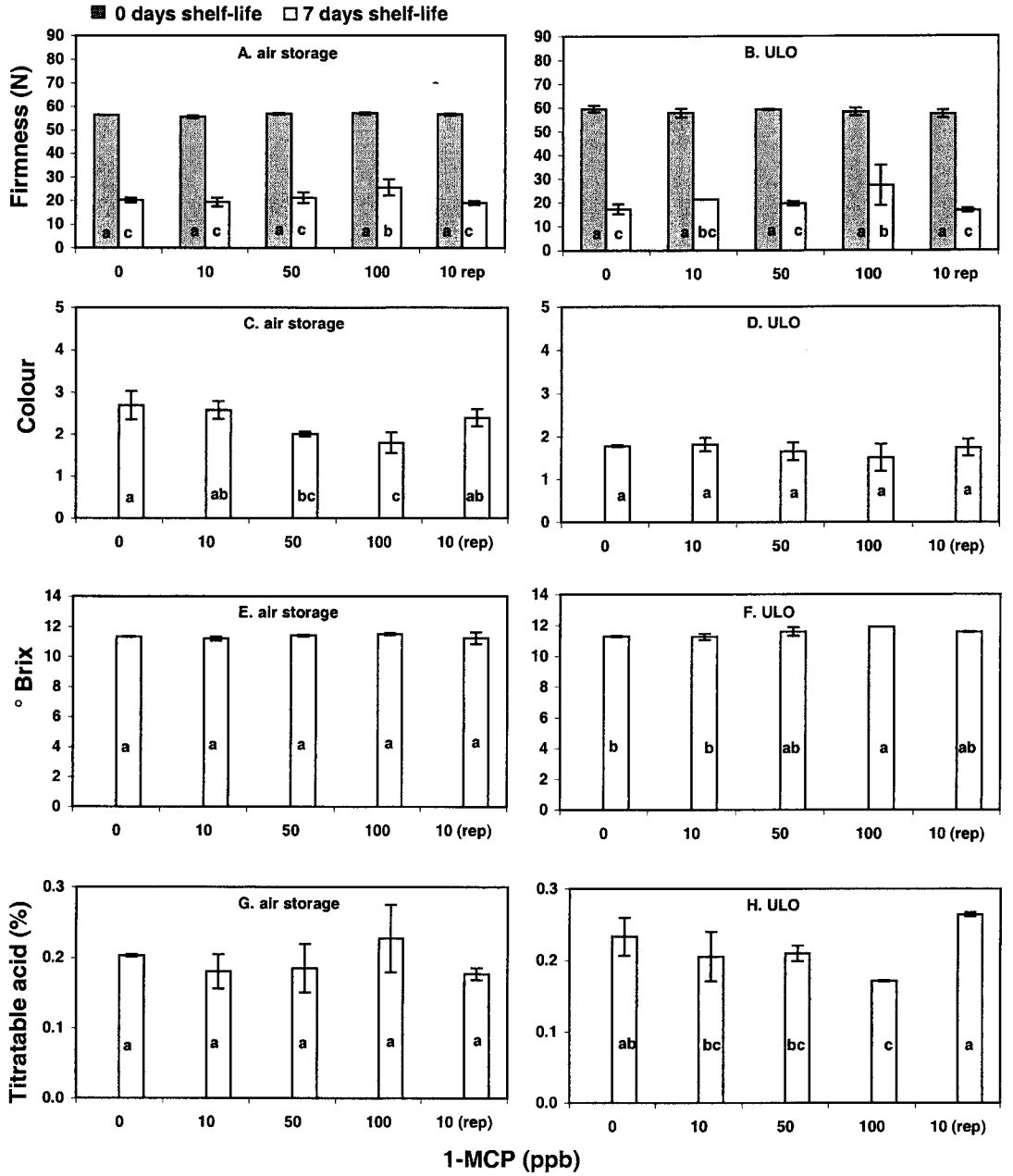

Fig. 4. Quality characteristics of Conference after 8 months of air storage (left) or ULO storage (right). 10 rep means a repeated treatment with 10 ppb 1-MCP. Vertical bars indicate mean ± std of two replicates. Columns within a chart marked with a same letter are not statistically different (P=0.05).

4.6 Disorders

A high percentage of pears with Brown-Heart was found after 4, 6 and 8 months ULO storage (Table 2). No Brown-Heart was found after air storage.
In general, the 50 and 100 ppb 1-MCP treatment promoted the development of Brown-Heart. Highest percentages of Brown-Heart were found for the 10 ppb replicated treatment. It is not clear whether this is the effect of 1-MCP or due to the way of treatment (temporarily interruption of ULO).

Apparently pears were very sensitive to the disorder. This is probably the consequence of the small harvest in The Netherlands in 2001. Pears were relatively large and were picked from trees with only few pears.

Disorders other than Brown-Heart (rot) were only scarcely found, with no effect of 1-MCP concentration.

Table 2. % of pears with Brown-Heart (ULO storage).

storage:	2 months	2 months	4 months	4 months	6 months	6 months	8 months	8 months
Shelf-life:	0 days	7 days	0 days	7 days	0 days	7 days	0 days	7 days
0 ppb	0	0	?	30	43	25	30	20
10 ppb	0	0	?	28	45	33	40	23
50 ppb	0	0	?	40	38	38	45	43
100 ppb	0	0	?	53	68	43	45	40
10 ppb, rep.	0	0	?	63	55	55	73	68

5 Conclusions

General
Treatment with 1-MCP can prolong storage life of Conference pears. Clear positive effects of 1-MCP were found on firmness retention during shelf-life. In some cases, 1-MCP inhibited yellowing and led to lower acidity. Pears in this experiment were sensitive to the disorder Brown-Heart as was shown by the high % of control pears with this problem. The incidence of Brown-Heart was stimulated by 1-MCP.
The applied 1-MCP dose after harvest is very important as pears must not be to soft and not be to firm at the time of consumption. The 50 ppb treatment had positive effects after 2-6 months storage but was not effective anymore after 8 months storage. The 100 ppb treatment was still effective after 8 months storage but led to relatively big differences between pears (high standard deviation of mean firmness). It will be difficult to decide upon the best concentration shortly after harvest, because at this moment the desired storage time is often not known yet. Also differences between years (ripening stage of pears) regarding the optimal 1-MCP concentration cannot be excluded.

Firmness
Although there seemed to be a small effect of 10 ppb 1-MCP after 2 months air storage, the 10 ppb treatment was never sufficient for good firmness retention. There was no effect of the repeated 10 ppb treatment each 2 months.
After 2 months of storage, the 100 ppb treatment prevented softening completely. The 50 ppb treatment strongly inhibited firmness loss, probably leading to good edible pears.
The MCP effect decreased with longer storage duration. After 8 months storage + shelf-life, only the 100 ppb treatment led to pears that were firmer than control pears. However, standard deviation was high. This probably reflected different ripening stages of pears at the time of treatment.

Colour
There was never an effect of 10 ppb 1-MCP. Treatment with 50 and 100 ppb inhibited yellowing after 2 and 4 months storage (air and ULO) and after 6 and 8 months storage (only air).

Soluble solids content
In general, there were no clear effects of 1-MCP on soluble solids content.

Titratable acidity
Titratable acidity was sometimes smaller at 1-MCP treated pears compared to control (50 ppb and 100 ppb + 2 months ULO, 100 ppb + 8 months ULO).

Disorders
Severe incidence of Brown-Heart occurred in control pears during ULO storage. The problem increased by treatments with 1-MCP.