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Research Note

1-Methyl cyclopropene extends postharvest life of spinach leaves

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ABSTRACT

Senescence of detached spinach leaves either untreated or treated with 0.1 or 1.0 μL L^{-1} 1-MCP has been investigated. 1-MCP treated leaves had higher chlorophyll content and photosystem II potential quantum yield (Fv/Fm) and lower solute leakage than untreated leaves after storage in darkness at 23 °C for 6 d, indicating a delay of senescence. Ethylene production was increased in spinach supplemented with 1-MCP after 3 d storage and then declined to the rates of untreated leaves. 1-MCP treated spinach had higher ascorbic acid and glutathione concentrations, and a low oxidised/reduced ratio for both antioxidants. Accumulations of ammonium and protein degradation were reduced by 1-MCP. The results presented here indicate that inhibition of ethylene sensitivity can be successfully used to extend the postharvest life of spinach leaves.

1. Introduction

Spinach is an important dietary leafy vegetable with high vitamin and mineral contents. To be accepted by consumers, leaves must be green and turgid. However, these attributes of acceptable produce are lost quickly during postharvest handling as a consequence of senescence, especially under non-refrigerated and/or dark conditions. Chlorophyll, protein, and antioxidant losses are characteristic changes taking place during leaf senescence, and these changes are under environmental and hormonal control (Ferrante and Francini, 2006). Previous studies showed that ethylene stimulates foliar senescence (Yamauchi and Watada, 1991). In particular, treatment of detached spinach leaves with ethylene accelerates senescence and enhances degradative processes (Hodges and Forney, 2000). Detached spinach leaves present two peaks of ethylene biosynthesis, the first peak is observed after excision from the plant and is smaller than the second and main peak occurring after 5–7 d of storage in the dark at 25 °C (Philosoph-Hadas et al., 1989). Treatment with an inhibitor of ethylene synthesis delayed the progress of spinach senescence (Philosoph-Hadas et al., 1991).

Several compounds interact specifically with ethylene receptors blocking plant responses to this hormone. Among them, 1-methylcyclopropene (1-MCP) has been successfully used at low concentrations to inhibit fruit ripening (Sisler and Serek, 1999). Most studies on the effects of 1-MCP were carried out with fruits or flowers, and only a few with leafy vegetables (Watkins, 2006, and references therein). These works show that the senescence of green tissues, an undesirable process in leafy vegetables, is delayed by treatments with 1-MCP.

The aim of this work was to assess the effects of 1-MCP treatments on postharvest life of spinach leaves.

2. Materials and methods

2.1. Plant material and treatments

Spinach plants (*Spinacia oleracea* L cv Bison) were collected from a local producer and mature leaves were used for the experiments. Leaves were exposed to 0.0 (untreated), 0.1 and 1.0 μL L^{-1} 1-MCP (Smart Fresh^{SM}) by incubation in 40 L air tight chambers at 23 °C for 6 h. Then leaves were stored at 23 or 4 °C in low density polyethylene bags. Samples were taken at harvest and after 3 and 6 d of storage in the experiments at 23 °C or weekly in the experiments at 4 °C.

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2.2. Leaf senescence parameters

Chlorophyll content was measured with a SPAD-502 Chlorophyll Meter (Minolta, Japan) and results expressed as SPAD units. Photosystem II potential quantum yield (Fv/Fm) and solute leakage were determined as previously described (Gómez et al., 2008). Ethylene production was measured by incubating leaves in glass flasks sealed with rubber caps for 1 h and ethylene determined with a KNK-3000-HGRC (Konik) gas chromatograph, fitted with an alumina column and a flame ionization detector (Bartoli et al., 1996).

2.3. Antioxidant contents

Reduced and oxidised ascorbic acid (AA and DHA, respectively) were measured by HPLC as described in Bartoli et al. (2006). Reduced and oxidised glutathione (GSH and GSSG, respectively) were determined as in Griffith (1980) with minor modifications as reported before (Gómez et al., 2008).

2.4. Nitrogen-containing compounds

For the determination of ammonium content, four leaf discs (fresh weight of 140–160 mg), were ground in 3 mL of water (HPLC grade) on an ice bath, and centrifuged at 12,000 × *g* for 10 min at 4 °C. Then 0.1 mL of the supernatant were mixed with 0.4 mL water, 2.5 mL 1% phenol, 0.005% sodium nitroprusside and 2.5 mL 0.5% NaOH, 0.84% Na hypochlorite and vigorously mixed with a vortex shaker. The mixture was incubated at 30 °C for 15 min, and absorbance was measured at room temperature at 635 nm (Beecher and Whitten, 1970). The calibration curve was prepared using ammonium sulfate as standard.

Three leaf discs (fresh weight of 100–120 mg), were ground in 0.5 mL homogenization buffer (62.5 mmol L^{-1} Tris–HCl pH 6.8, 10% glycerol, 0.05% (v/v) mercaptoethanol) plus 3 μL of protease inhibitor cocktail (Sigma–Aldrich, Inc.) on ice, and centrifuged at 12,000 × *g* at 4 °C for 10 min. The protein content was estimated in the supernatant fraction by running aliquots in SDS–PAGE gels and scanning the gels after Coomassie blue staining. The scanned gels were quantified with the SigmaGel software (v. 1.0, Jandel Scientific), and the signals corresponding to all the proteins in each sample were added to estimate the concentration of protein in each sample (Bartoli et al., 2004).

2.5. Statistical analysis

Data are presented as the average of four independent experiments and analyzed by means of ANOVA. The means were compared by the LSD test at a significance level of 0.05.

3. Results and discussion

3.1. Effects of 1-MCP on leaf senescence

Leaf senescence was followed by measuring chlorophyll content, PSII potential quantum yield (Fv/Fm), solute leakage and ethylene production (Fig. 1A–D). Small decreases in chlorophyll content and Fv/Fm in untreated leaves on day 3 were prevented by treatment with 1.0 μL L^{-1} 1-MCP (Fig. 1A and B). At a later stage in leaf senescence (6 d after harvest) 1.0 μL L^{-1} 1-MCP delayed this

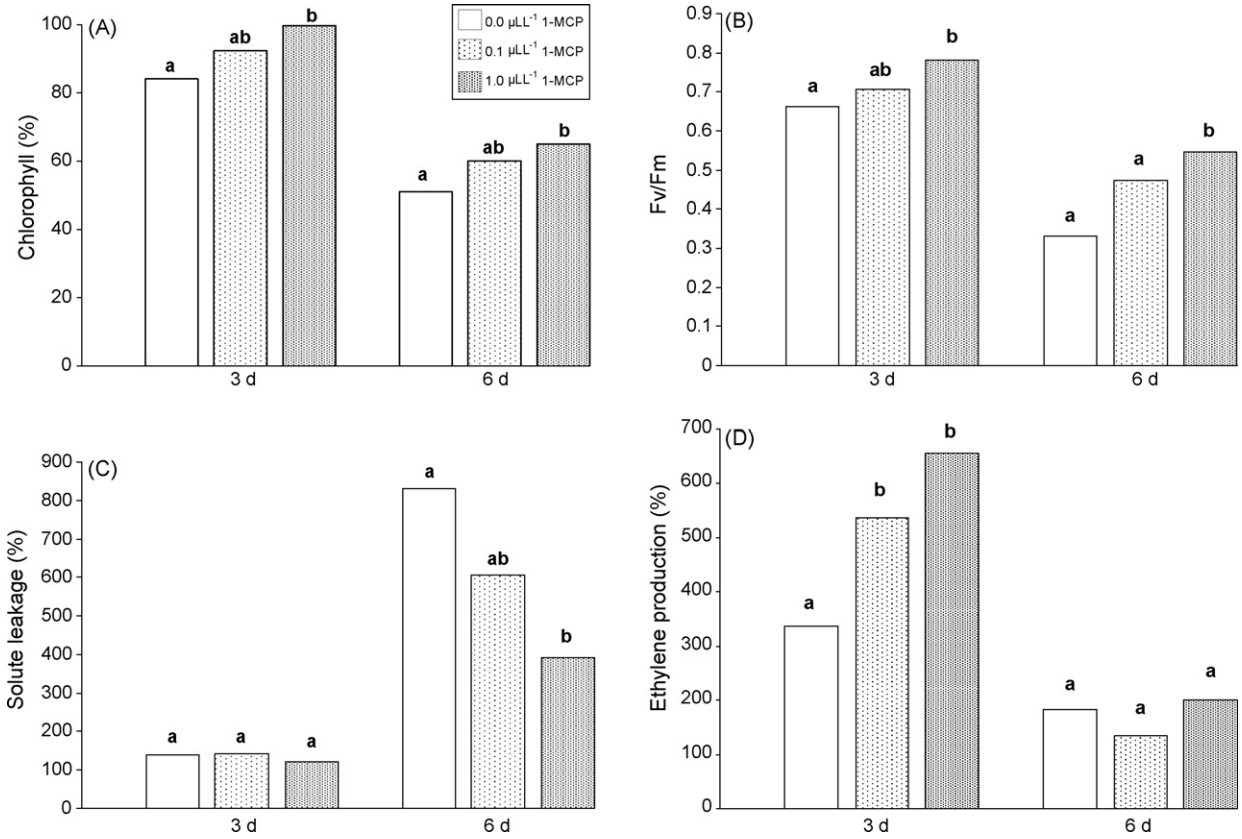

Fig. 1. Chlorophyll content (A), Fv/Fm (B), solute leakage (C) and ethylene production (D) during dark-induced senescence of 1-MCP treated spinach leaves. Leaves were untreated, or treated with 0.1 and 1.0 μL L^{-1} 1-MCP by incubation in air tight chambers for 6 h and thereafter stored in a dark chamber at 23 °C. Chlorophyll content, solute leakage and ethylene production are expressed as a % of values at harvest: 34.2 SPAD units, 30 μS m^{-1} kg^{-1} s^{-1} and 0.6 μL kg^{-1} h^{-1}, respectively. At harvest Fv/Fm was 0.88. Values are the means of four independent experiments with at least three replicates each. Means followed by the same letters represent a statistically homogenous group on the same sampling day (ANOVA $P \leq 0.05$).

process, as shown by higher values of chlorophyll content and Fv/Fm, and a decrease in solute leakage (Fig. 1A–C). Treatment with the lower 1-MCP concentration (0.1 μL L^{-1}) had no effect on any of these senescence parameters. A second treatment with 1-MCP on day 3 did not delay postharvest senescence beyond the effect of a single treatment on the day of harvest (data not shown). Ethylene production increased by 60 and 95% with 0.1 and 1.0 μL L^{-1} 1-MCP, respectively, after 3 d of dark storage. However, similar leaf ethylene production was observed between treated and untreated samples after 6 d storage. The increase of ethylene production induced by 1-MCP was previously observed for other leafy vegetables (Lomaniec et al., 2003; Jiang et al., 2002).

Low temperatures (0–5 °C) have been demonstrated to be the best storage condition for the preservation of vegetables, included spinach (Cantwell and Kasmire, 2003, and references therein). Detached spinach leaves showed the first signs of deterioration after 2 weeks of storage in darkness at 4 °C (data not shown). Treatment with 1-MCP at harvest significantly delayed senescence of leaves stored at low temperature for 4 weeks (Table 1).

Application of 1-MCP has been successfully used to extend postharvest life of parsley or coriander leaves at 20–25 °C (Lomaniec et al., 2003; Jiang et al., 2002), but 1-MCP failed to delay senescence at 5 °C (Jiang et al., 2002). Exogenous application of ethylene accelerates spinach senescence, thus demonstrating the sensitivity of this species to the hormone (Hodges and Forney, 2000; and supplementary material Table 1). The delay of leaf senescence by 1-MCP application shows that endogenous ethylene is involved in spinach senescence, even when leaves are stored at low temperature.

Table 1
Effect of 1-MCP supplementation in spinach leaf senescence under refrigerated conditions. Chlorophyll content, PSII quantum yield (Fv/Fm) and solute leakage in spinach leaves treated with 1.0 μL L^{-1} 1-MCP at harvest. Leaf senescence parameters were measured after storage in darkness at 4 °C for 4 weeks. Chlorophyll and solute leakage are expressed as a % of values at harvest: 39.8 SPAD units and 19.0 μS m^{-1} kg^{-1} s^{-1}, respectively. At harvest Fv/Fm was 0.85.

Treatment	Chlorophyll (%)	Fv/Fm	Solute leakage (%)
Untreated	76.6 a	0.72 a	210 a
1.0 μL L^{-1} 1-MCP	92.8 b	0.82 b	105 b

Different letters indicate statistically significant differences (ANOVA $P \leq 0.05$).

3.2. Effects of 1-MCP on antioxidants

The contents of both AA and GSH decreased during storage at 23 °C (Fig. 2A and B). On day 3, AA and GSH levels were only 23 and 63% of those measured at harvest (day 0) in control leaves. The decreases in AA and GSH contents were attenuated by 1-MCP. At the end of the storage period (6 d), both AA and GSH showed the lowest values compared to those at harvest (6 and 35%, respectively) but

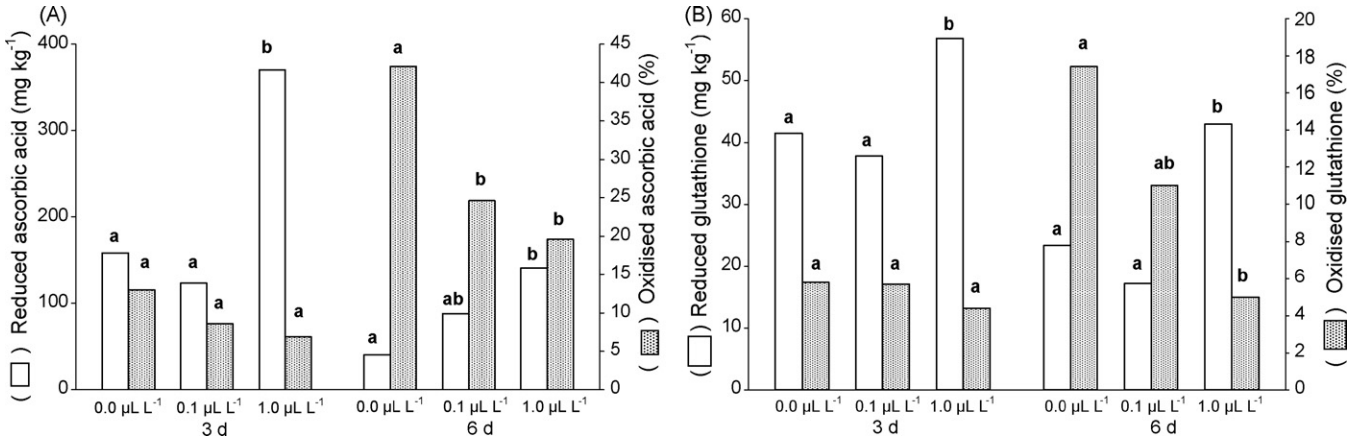

Fig. 2. Contents and redox state of antioxidants during dark-induced senescence of 1-MCP treated spinach leaves: AA, DHA (A) and GSH, GSSG (B). Leaves were untreated or treated with 0.1 and 1.0 μL L^{-1} 1-MCP by incubation in air tight chambers for 6 h and thereafter stored in a dark chamber at 23 °C. Reduced antioxidant contents at harvest were 686.8 and 64.5 mg kg^{-1} for AA and GSH, respectively. The oxidised/reduced antioxidant ratios at harvest were 8.2 and 4.5% for DHA/AA and GSSG/GSH, respectively. Values are the means of four independent experiments with at least three replicates each. Means followed by the same letters represent a statistically homogenous group on the same sampling day (ANOVA $P \leq 0.05$).

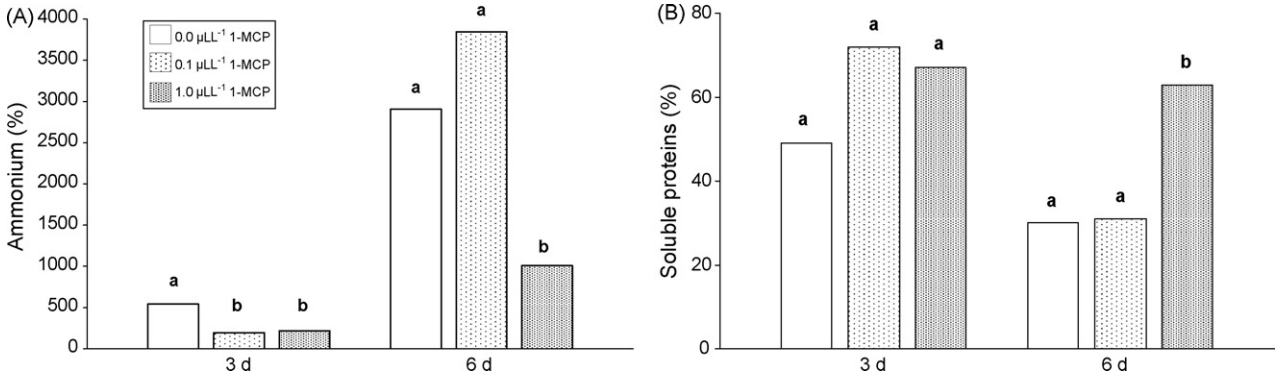

Fig. 3. Contents of ammonium (A), and soluble proteins (B) during dark-induced senescence of 1-MCP treated spinach leaves. Leaves were untreated or treated with 0.1 and 1.0 μL L^{-1} 1-MCP by incubation in air tight chambers for 6 h and thereafter stored in a dark chamber at 23 °C. Results are expressed as a % of values at harvest: 35.00 μg kg^{-1} and 6.64 g kg^{-1} for ammonium and soluble protein contents, respectively. Values are the means of four independent experiments with at least three replicates each. Means followed by the same letters represent a statistically homogenous group on the same sampling day (ANOVA $P \leq 0.05$).

this trend was partially reversed by 1.0 μL L^{-1} 1-MCP (20 and 66% of their contents at harvest, respectively for AA and GSH). The DHA/AA ratio increased after 3 and 6 d, but this increase was prevented by 1-MCP treatments (Fig. 2A). On the other hand, the GSSG/GSH ratio increased after 6 d storage period, and this was partially reverted by 1.0 μL L^{-1} 1-MCP (Fig. 2B). In a previous work, heat shock was successfully applied to extend postharvest spinach life (Gómez et al., 2008). Although the treatment did not prevent the decrease in antioxidant contents, the ratios of oxidised/reduced forms were kept low. In spinach, the senescence-associated decrease in antioxidant contents was accelerated by exogenous ethylene application (Hodges and Forney, 2000). These results suggest that antioxidant redox state might be important for modulating the rate of leaf senescence.

3.3. Effects of 1-MCP on nitrogen-containing compounds

Leaf ammonium content increased 5.4-fold between days 0 and 3 of dark-induced senescence at 23 °C. Treatments with 0.1 or 1.0 μL L^{-1} 1-MCP reduced this increase on day 3, whereas only the higher concentration of 1-MCP reduced ammonium accumulation on the sixth day of storage (Fig. 3A). Protein degradation in detached leaves is the main source of ammonium during senescence (Mattsson and Schjoerring, 2003), therefore, protein levels were determined. The leaf soluble protein content of untreated leaves decreased by 51 and 70% after storage for 3 and 6 d, respectively (Fig. 3B). Inhibition of ethylene action prevented this loss. Leaves treated with 1.0 μL L^{-1} 1-MCP retained 2-fold higher protein content than untreated leaves, on the sixth day. The reduced accumulation of ammonium in 1.0 μL L^{-1} 1-MCP treated samples may be due to inhibition of protein degradation.

4. Conclusions

The results of this work suggest that a single treatment with 1.0 μL L^{-1} 1-MCP for 6 h can be used to extend the postharvest storage life of spinach, and to maintain a better nutritional value (e.g., higher vitamin and protein contents) and appearance (greenness).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.postharvbio.2009.10.004.

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