Methyl Jasmonate Extends Shelf Life and Reduces Microbial Contamination of Fresh-Cut Celery and Peppers

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During a study of the ameliorative effects of methyl jasmonate (MJ) on chilling injury to vegetables, a decrease in the rate of deterioration of treated fresh-cut segments was noticed, along with an apparent decrease in microbial growth. This study showed that MJ vapor from a 10^{-4} or 10^{-5} mol source in a 1 L container retarded deterioration of celery sticks for 2 weeks at 10 °C. The number of bacterial colonies was reduced to 1/1000 of control after 1 week of storage. A MJ emulsion applied as a dip at 10^{-4} or 10^{-5} mol/L retarded deterioration of green pepper strips for 2 weeks at 10 °C. The number of bacterial colonies was reduced to 1/1000 of control also after 1 week of storage and, in particular, the appearance of soft rot was retarded by the jasmonate treatment. Measurement of the amount of MJ vaporized during storage was done by use of a [^{3}H]jasmonate internal standard. Less than 1×10^{-6} mol of MJ vapor was necessary to cause the biochemical changes in the stored vegetables resulting in prolonged storage life and decreased microbial growth.

Keywords: *Celery; green peppers; methyl jasmonate; microbial contamination*

INTRODUCTION

Enhanced resistance to chilling temperatures and decreased incidence of injury to temperature-sensitive fruits and vegetables such as zucchini squash (Wang and Buta, 1994), bell peppers, and avocado (Meir et al., 1996) resulted from timely applications of methyl jasmonate (MJ) as a vapor or emulsion prior to exposure to low temperatures. These responses were several of many effects resulting from applications of MJ or other jasmonic acid derivatives to plant material. Jasmonates occur widely in the plant kingdom as the free jasmonic acid and the epi form, their methyl esters, and many conjugates such as with amino acids. The free acids and methyl esters affect plant growth and development, as well as having roles in the response to other stresses such as wounding, pathogen attack, and desiccation (Koda, 1992; Sembdner and Parthier, 1993). MJ was shown to be involved in interplant communication of stresses (Farmer and Ryan, 1990). The ease of use of the commercially available MJ esters (a mixture of isomers) allowed the initial discovery of the enhanced resistance to chilling injury to be made. Knowledge of the effectiveness of the compound in interplant communications suggested that further study of low but nonchilling temperature stress responses of fruits and vegetables should be done utilizing the volatility of MJ.

During subsequent investigations of the effects of MJ treatments as a vapor or an emulsion dip during low-temperature storage of several fruits and vegetables, a decrease in the rate of deterioration of fresh-cut segments of the plant material was noticed along with an apparent decrease in microbial growth. Several experiments have been designed to investigate whether the application of MJ might extend the storage life of fresh-cut celery and bell peppers by decreasing the rate of deterioration of quality and inhibiting microbial growth during low-temperature storage and to better determine the quantities of exogenously applied MJ available for use as a signaling compound.

MATERIALS AND METHODS

Plant Material. Celery and green bell peppers were obtained from a local market. Care was taken to select only fresh produce with no indications of chilling stress.

Reagents. Methyl (±)-jasmonate was obtained from Aldrich Chemical Co., Milwaukee, WI. [2,3-^{3}H]-2-Jasmonic acid was obtained from American Radiolabeled Chemicals, St. Louis, MO.

Bioassay. Assays were carried out in clear polystyrene containers of 1 L capacity with snap-on lids that were used for fresh-cut produce sales in a local market. For experiments using MJ as a vapor, 2.0 (10^{-5} mol) and 22.0 μL (10^{-4} mol) were applied to a glass fiber sheet suspended from the lid of the container holding the vegetable pieces to be treated. For experiments using MJ emulsions at 10^{-4} or 10^{-5} M, the compound was placed in Tween 20 surfactant (final concentration = 0.05%) and diluted to the final volume with distilled H_2O. The vegetable pieces were immersed in the emulsions for specified times.

Celery stalks were cut into segments of 7 cm length and then rinsed only with distilled H_2O because low surface populations of bacteria had been found in preliminary assays. Ten pieces were placed in each of four 1 L containers for each experiment; the containers were then placed in storage at 10 °C, and three experiments using MJ vapor were conducted. Green bell peppers were surface sterilized by immersion in a 5% Clorox solution before being cut because of high surface populations of soft rot bacteria, rinsed with distilled water, and cut into longitudinal strips of 1 cm width. In addition to experiments using MJ vapor treatments, pepper strips were immersed in treatment emulsions for 2 min and then drained in a colander for 1 min. Strips used as controls were immersed in distilled H_2O and drained since in previous experiments no effect of Tween 20 on bacterial proliferation had been found with this surfactant concentration. Twenty randomized strips were placed in each of two containers for each experiment; three experiments were conducted with storage at 10 °C. Visual observations of the occurrence of browning of all of the

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strips in the experiments were made by two individuals. Samples were taken at intervals from the containers in storage for microbial assay. Atmospheric changes in the containers due to respiration were not considered significant at the low storage temperature.

Microbial Assay. One gram tissue samples cut from the ends of celery pieces and pepper strips were rinsed with sterile distilled H_2O. The samples were weighed in a sterile beaker and suspended in sterile distilled water to achieve a 10-fold (w/v) dilution of the sample. Samples were vortex mixed for 30 s to suspend microbes present on the cut surfaces. The rinse solutions were then dilution plated onto 2% Difco nutrient agar plates. The inoculated plates were incubated for 48 h at 20 °C, and visible colonies were counted. Microbial populations were determined at the time the produce was cut prior to treatment, 2 h after treatment, 1 week after treatment, and 2 weeks after treatment. At least three pieces were assayed at each interval from control and treated samples. Statistical analyses of the microbial assays were done using the Kruskal−Wallis one-way analysis of variance with pairwise multiple comparison procedures using Dunnett's method.

Quantification of MJ Levels in the Headspace. Methyl [2,3-^{3}H]-2-jasmonate was synthesized for use as an internal standard by esterification of [2,3-^{3}H]- 2-jasmonic acid combined with jasmonic acid by treatment with diazomethane in ether. The conversion to MJ was 98% as determined by thin-layer chromatography and subsequent tritium counting. The MJ containing [^{3}H]jasmonate (10^{-4} mol) was then applied to the glass fiber sheet source in the 1 L polystyrene container with the snap-on lid. Containers with and without celery segments were prepared. Vapor samples were taken at different intervals during storage by insertion of a 10 mL syringe into a port in the lid of the container, and then the sealed syringe sample was cooled rapidly. Levels of MJ vapor present were obtained by rinsing the condensed compound from the syringe and determining ^{3}H quantities in a scintillation counter. Quantities of MJ that had volatilized and then condensed on the surfaces of the containers as well as on the surface of the celery sticks were determined after washing of surfaces with dichloromethane, removal of the solvent at room temperature, and subsequent measurement in a scintillation counter. Two experiments were performed, and the values obtained were averaged.

RESULTS AND DISCUSSION

The effects of MJ vapor on the proliferation of the bacterial flora of celery stalks are presented in Figure 1. Bacterial proliferation was retarded significantly by the vapor obtained from the 1×10^{-4} mol MJ source to colony levels 1/1000 of those in nontreated tissue after 1 week in storage at 10 °C, with a decrease in effectiveness by the second week of storage such that no significant difference from bacterial levels in nontreated tissue was found. The vapor from the 1×10^{-5} mol source was somewhat more effective and reduced bacterial proliferation significantly to colony levels 1/1000 of those in nontreated tissue after 1 week; a comparable amount of reduction was found after 2 weeks of storage. Accompanying the decrease in bacterial proliferation was an inhibition of celery stalk discoloration (browning) during cold storage as shown in Table 1. MJ vapor from the 1×10^{-4} mol source inhibited browning of the stalks during the first week of storage. The cumulative inhibitory effect was more pronounced in the second week. A similar inhibition of browning was found with the MJ vapor from the 1×10^{-5} mol source; however, the amount of discoloration increased in one of the three experiments during the second week of storage. This variability may in part be due to differences in the response of the particular sample to uptake of MJ vapor during each of our treatments. The use of the vapor

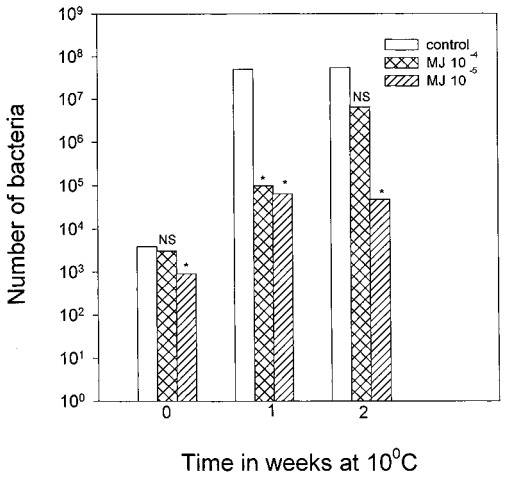

Figure 1. Comparison of bacterial populations on fresh-cut celery sticks at three sampling dates during 2 weeks of storage at 10 °C following treatment with 10^{-4} and 10^{-5} mol of MJ vapors with nontreated controls in l L containers. C, nontreated control; MJ 10^{-4}, 10^{-4} mol jasmonate treatment; MJ 10^{-5}, 10^{-5} mol jasmonate treatment. NS indicates that the number of bacteria was not different from the control at the sampling date; an asterisk indicates that there was a significant difference between the numbers of bacteria on the control and on the treated tissues, $P \leq 0.05$.

Table 1. Inhibition of Browning on Cut Surfaces of Celery by MJ

time (10 °C; weeks)	treatment		
	control	MJ 10^{-4} mol	MJ 10^{-5} mol
1	0.59[a]	0.26	0.30
2	2.15	0.75	1.46

[a] Visual observations of browning of cut surfaces of celery pieces were made on a 0−5 scale, where 0 = no browning, 1 = very slight discoloration, 2 = slight browning, 3 = light to moderate browning, 4 = moderate browning, and 5 = dark browning. Average values of three experiments are shown.

was an attempt to employ the least invasive means of application of the jasmonate to a nonsterilized vegetable because immersion in an emulsion could contribute to enhanced decay.

Bacterial proliferation was reduced on strips of surface-sterilized green bell pepper after immersion in MJ emulsions (Figure 2) but not during MJ vapor exposure (data not shown). Immersion of the pepper slices in 1×10^{-4} M MJ retarded bacterial proliferation significantly after 1 week of storage with a decrease in effectiveness during the second week of storage in two of the three experiments such that there was no longer a significant difference from colony levels in the controls. Treatment of the pepper slices with 1×10^{-5} M MJ resulted in a significant decrease in bacterial colony levels after both 1 and 2 weeks storage. Apparently higher concentrations of MJ applied as an emulsion were necessary to reduce bacterial proliferation on the pepper strips than on the celery stalks, where no volatilization of the compound was detected gravimetrically during the experiments. The effects of MJ in decreasing the deterioration of the pepper slices during cold storage are summarized in Table 2. MJ treatments slowed the loss of green coloration (chlorophyll) and the onset of tissue browning, as well as decreasing the amount of tissue softening by the second week of storage. It should be noted that treatment with the higher concentration, 1×10^{-4} M MJ, resulted in more deterioration of the green pepper strips during storage

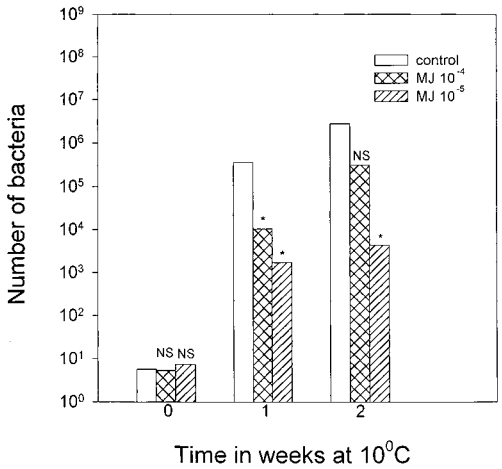

Figure 2. Comparison of bacterial populations on fresh-cut pepper strips at three sampling dates during 2 weeks of storage at 10 °C following dipping in 10^{-4} and 10^{-5} M MJ emulsion with nontreated controls. C, nontreated control; MJ 10^{-4}, 10^{-4} M jasmonate treatment; MJ 10^{-5}, 10^{-5} M jasmonate treatment. NS indicates that the number of bacteria was not different from the control at the sampling date; an asterisk indicates that there was a significant difference between the numbers of bacteria on the control and on the treated tissues, $P \leq 0.05$.

Table 2. Effects of MJ[a] on Deterioration of Green Pepper Strips during Subsequent Storage at 10 °C

treatment	symptoms[b]
water control	severe bacterial soft rot, fungal growth, browning, and loss of chlorophyll
10^{-4} M MJ	fungal growth, slight bacterial soft rot, slight loss of chlorophyll
10^{-5} M MJ	no deterioration in two of three experiments, one strip with slight bacterial soft rot in third experiment

[a] MJ was applied in emulsion with Tween 20. [b] Symptoms observed during 3 weeks of storage at 10 °C.

than treatment with the lower concentration. This effect could be considered an example of the promotion of senescence by MJ reported by Ueda et al. (1981) when applied at 1×10^{-5} M in bioassays. Several more recent findings reviewed by Koda (1992) indicated a role for MJ in the enhancement of chlorophyll degradation in leaf tissue due to an increase in proteolytic and lipolytic activities if treatment concentrations such as 5×10^{-5} M were maintained. This effect on enhancement of tissue senescence may explain also the variability in control of bacterial proliferation in celery by vapor obtained from treatment with 1×10^{-4} M MJ described earlier. Although we did not identify the bacterial microflora present on the pepper strips, we can infer from our observations of decreased bacterial softening of treated strips that our treatment reduced the population of soft rotting bacteria in these strips.

The quantity of MJ vapor that was available for utilization to cause the many biological responses has not been determined in almost all published studies, where usually several microliters of MJ was placed in a container and volatilization allowed to occur. The calculated atmospheric MJ levels at 24 °C were assumed to be 2.4×10^{-7} mol/L at the initiation of the experiment in the one study where MJ levels were discussed (Franceschi and Grimes, 1991). To be able to measure MJ vapor levels derived from an exogenous source at various times during the experiments involving placement of vegetables into cold storage, a means of measuring levels using an internal standard was devised. [^{3}H]MJ was incorporated into the quantities of MJ employed in the treatments. This allowed for a better determination of the quantities of exogenous MJ available for utilization as a signaling compound to be made at several times during storage of the celery sticks in the sealed container (Table 3). Almost nondetectable levels of MJ vapor (1×10^{-8} mol) were found in the container with the celery sticks when measurements were made during the course of the experiment. However, very low MJ levels ($>10^{-7}$ mol) were found on both the surfaces of the celery sticks and the container, indicating that MJ volatilization had occurred during the storage period. Endogenous levels of jasmonic acid were reported to be in the 0.1−0.3 nmol/g of fresh weight range for leaf tissue of several crop plants (not celery or green pepper) for which analyses have been done (Farmer, 1994); these levels would be equivalent to (2−4) $\times 10^{-8}$ mol MJ in the celery stick sample that was then exposed to an excess quantity of exogenous MJ vapor during storage. Uptake of even a small portion of the excess exogenous MJ was sufficient to induce metabolic changes resulting in the inhibition of bacterial proliferation and a decrease in related degradative effects.

Table 3. Utilization of MJ during Storage[a]

	container with celery sticks[b]	empty container
initial MJ/source	1×10^{-4} mol	1×10^{-4} mol
MJ vapor 2 h/18 °C	1×10^{-8}	1×10^{-7}
MJ vapor 3 days/10 °C	1×10^{-8}	1×10^{-7}
MJ vapor 7 days/10 °C	1×10^{-8}	1×10^{-8}
MJ on surfaces 7 days/10 °C	5×10^{-7} mol[c]	5×10^{-7} mol

[a] MJ contained [^{3}H]MJ as an internal standard. Data shown are the average of two experiments. [b] Container volume was 1 L. [c] Celery surface wash was included.

The inhibition of bacterial growth in culture by MJ in the absence of plant material was investigated recently by I. Babic (Horticultural Crops Quality Laborary, USDA-ARS, Beltsville, MD, 1997, personal communication). Several bacterial species, including *Listeria monocytogenes*, were cultured in the presence of MJ, 1-octanoic acid, and 2-hexenal at 37 °C. MJ, as well as the other two compounds, inhibited bacterial growth only when relatively high concentrations such as 1×10^{-3} M were used. Similar inhibitory concentrations for the growth of other bacteria had been reported using hexenal and other six-carbon volatile compounds derived from plant lipid peroxidation by the lipoxygenase/hydroperoxide lyase pathway (Deng et al., 1993).

Jasmonic acid, and derivatives such as MJ, have been described as signaling compounds that stimulated the expression of wound-inducible and defense-related genes, as well as being involved in many developmental processes in plants (Farmer, 1994). The jasmonates were produced from linoleic or linolenic acids via the lipoxygenase/hydroperoxidase pathway for the modification of fatty acids (Vick and Zimmerman, 1987). The response of plant tissues to environmental stresses has been shown to involve transitory increases in jasmonate levels, which resulted in induced expression of phenylalanine ammonia-lyase genes involved in the chemical defense mechanisms of plants against pathogens (Peña-Cortés et al., 1995; Gundlach et al., 1992). Volatile MJ induced the accumulation of wound-inducible proteinase inhibitor proteins in tomato plants (Farmer and Ryan, 1990). Airborne MJ also stimulated an increase in the

natural furanocoumarin levels in the leaves of celery plants (Miksch and Boland, 1996). This was considered as indicative of increases in the chemical defenses of the plant. The findings that pretreatment of plant material with MJ prior to exposure to cold temperature stress resulted in decreased chilling injury indicated that biochemical defense mechanisms against environmental stress were activated (Wang and Buta, 1994). This pretreatment could have also activated the plant defense mechanisms effective against pathogens such as bacteria. The observed suppression of bacterial proliferation in celery stalk segments and green pepper strips during low-temperature storage suggests that the inherent chemical defense mechanisms of the plant tissues were able to be activated sufficiently by low-concentration jasmonate treatments to lengthen the shelf life of the commodities. These jasmonate treatments may be a practical means of increasing food safety of fresh-cut fruits and vegetables by enhancing resistance to bacterial growth as well as decreasing the incidence of chilling injury.

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