1-Methylcyclopropene Counteracts Ethylene-Induced Microbial Growth on Fresh-Cut Watermelon

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ABSTRACT: The effects of exogenous ethylene, 1-methylcyclopropene (1-MCP), or both on microbial growth on watermelon fruit and watermelon slices were investigated. Freshly harvested seedless watermelons (*Citrullus lanatus*, cv. Sugar Heart) were treated with 0.5 or 1.0 ppm 1-MCP, 10 ppm ethylene, 1-MCP + ethylene, or left untreated as controls. Fruits were processed into wedge-shaped slices, packaged into rigid trays sealed with a polyethylene film with a 29.2 pmol s^{-1} m^{-2} Pa^{-1} oxygen transmission rate. The slices were evaluated after 0-, 6-, and 12-d storage at 5 °C. Ethylene treatment alone increased the populations of aerobic bacteria, lactic acid bacteria, and yeasts and molds on the packaged slices during storage compared to those on corresponding control slices and resulted in extensive juice leakage from the slices. The ethylene treatment also resulted in high aerobic bacterial counts throughout the flesh of whole melons compared to the controls. Treating watermelons with 0.5 or 1.0 ppm 1-MCP prior to ethylene exposure counteracted the deleterious effects of ethylene. Extending the time from harvest to 1-MCP treatment increased the population of aerobic bacteria, but had no detectable effect on the growth of lactic acid bacteria or yeasts and molds. The results indicate that low concentrations (0.5 or 1.0 ppm) of 1-MCP can be used on whole watermelon to avoid deleterious effects of exogenous ethylene to which the melons could be exposed during shipping or storage.

Keywords: 1-methylcyclopropene, 1-MCP, *Citrullus lanatus*, ethylene, watermelon

Introduction

The market for fresh-cut fruits increased significantly in the last decade. The latest available watermelon sales data (for the year 2003) indicate that fresh-cut watermelon accounted for 46% of the total watermelon sales that topped $1.5 billion (Natl. Watermelon Promotion Board 2003). Like other fruit, fresh-cut watermelon deteriorates faster than the whole fruit due to the wounding that occurs during processing (Watada and others 1990, 1996) as well as physiological changes that occur postprocessing (Karakurt and Huber 2004; Mao and others 2004, 2006). Although watermelon is a nonclimacteric fruit (Karakurt and Huber 2002) and usually produces little ethylene (Elkashif and others 1989), its physiology and quality are affected by low concentrations of exogenous ethylene to which the watermelon may be exposed during transportation and storage. For example, the ethylene produced by bananas stored nearby can have severe consequences for whole watermelons according to the Natl. Watermelon Promotion Board. Exogenous ethylene will induce placental tissue softening and water soaking, electrolyte leakage, rind softening, and enhanced phospholipid degradation in watermelons (Karakurt and Huber 2002; Mao and others 2004, 2006). These changes will also likely condition watermelon tissues to be more conducive to microbial growth, hastening the deterioration process.

The chemical 1-methylcyclopropene (1-MCP) is a potent antagonist to the action of ethylene and is useful in prolonging the quality of various fresh-cut fruits including apples (Jiang and Joyce 2002; Perera and others 2003) and pineapples (Budu and Joyce 2003). At low concentrations, 1-MCP appears to irreversibly bind to the ethylene receptors of plant tissues resulting in ethylene insensitivity and delaying or inhibiting ethylene-mediated ripening and senescence processes of various fruits (Blankenship and Dole 2003; Mao and others 2004). Although 1-MCP alone does not always maintain the quality of some fruit, especially nonclimacteric fruit such as watermelon (Ku and others 1999; Porat and others 1999; Pelayo and others 2003), 1-MCP can mitigate the deleterious effects of exogenous ethylene and thereby delay the ripening process (Mao and others 2004; Nilsson 2005). Since 1-MCP affects the ethylene-induced deterioration of watermelon (Mao and others 2004), 1-MCP will likely also affect the subsequent growth of microbes on watermelon fruit and slices.

The objectives of this study were to examine (1) the influence of ethylene, applied to whole watermelon, on the microbial populations and water leakage of subsequently processed fresh-cut watermelon stored in sealed packages at 5 °C and (2) the ability of 1-MCP to counteract ethylene-induced effects. The effect of ethylene on microbial populations of whole watermelons was also examined.

Materials and Methods

Fruit

Seedless watermelons (*Citrullus lanatus*, cv. Sugar Heart) were harvested at the commercial maturity stage by a major grower/packer in central Delaware, and immediately transported to the ARS Beltsville Agricultural Research Center in Maryland. Undamaged watermelons were selected to meet size uniformity and the selected fruit were stored at 20 °C until used for experimentation.

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Treatment with 1-MCP and ethylene

At 1 and 7 d after harvest, uniform sets of 20 watermelons were treated with 0.5 or 1.0 ppm 1-MCP gas for 18 h at 20 °C or left untreated. Subsets (10 fruit) of the control and the 1-MCP-treated melons were then subjected either to exogenous ethylene treatment (10 ppm) for 5 d at 20 °C or air storage. The melons were stored an additional 7 d in air at 20 °C prior to processing.

Processing of watermelons and storage of fresh-cut slices

The melons were rinsed with tap water followed by two 1-min dips with 100 ppm sodium hypochlorite (pH 6.5). With a sharp sanitized custom-made knife, four 4-cm wide rings were latitudinally cut from the center of the fruit. The rings were then processed into 6 equally sized wedge-shaped slices. The slices were randomized and placed (2 per container) in 13.5 cm × 19 cm × 4 cm rigid polypropylene trays (Pactiv Corp., Lake Forest, Ill, U.S.A.) and sealed with a 29.2 pmol s^{-1} m^{-2} Pa^{-1} OTR film (Package Concept Corp., Salinas, Calif, U.S.A.). The packaged slices were stored at 5 °C for subsequent evaluation. Six replications (melons; trays) per treatment were evaluated.

Microbial analyses of packaged watermelon slices

On days 0, 6 and 12, tissue samples (25 g) were taken from the melon slices and homogenized with 225 mL of sterile PBS (pH 7.0) at 260 rpm in a stomacher 400 Biomaster (Seward Limited, London, UK). The resultant slurry was filtered through sterile glass wool, serially diluted with PBS as necessary, and then plated in duplicate onto potato dextrose agar (PDA, Difco Lab, Sparks, Md., U.S.A.) supplemented with chloramphenicol (200 μg mL^{-1}), MRS (Difco Lab), and tryptic soy agar (TSA, Difco Lab) using a spiral plater (Wasp II Spiral Plater, DW Scientific, West Yorkshire, UK). After 24 (TSA) or 48 to 72 h (PDA-chloramphenicol) of incubation at 30 °C in air or 48 h (MRS) incubation at 30 °C under a modified atmosphere (MA: 20 kPa CO_2 and 5 kPa O_2), plates were read with an automated plate counter (ProtoCOL, Synoptics, Cambridge, UK). Yeast and mold counts from PDA-chloramphenicol plates, aerobic bacterial counts from TSA plates, and lactic acid bacterial counts from MA-incubated MRS plates are reported as log cfu g^{-1}.

Microbial analysis of whole watermelon treated with ethylene or air

Aerobic bacterial populations in control and ethylene-treated whole watermelons were examined after 7-d storage at 20 °C. Two fruits from each treatment were surface sanitized and aseptically quartered. A sterile 20-mm-dia cork borer was used to take a 100-mm-long core from the fruit center outward to the rind. Cores were segmented from the rind inward at depths of 10, 20, 40, and 100 mm. Samples were collected in triplicate, weighed, and homogenized as described above in the presence of 25 mL PBS, pH 7.4. Each homogenate was filtered through sterile glass wool and serially diluted for spiral plating onto duplicate TSA plates. Plates were incubated at 28 °C for 24 h and bacteria were enumerated as described above.

Juice leakage

Juice that had leaked from the watermelon slices during storage was collected and the amount was determined using a graduated syringe.

Statistical analysis

Data were analyzed using the General Linear Model procedure of SAS (SAS Institute Inc., version 9.1, Cary, N.C., U.S.A.). Means were separated with the Duncan's multiple range test or *t*-tests (LSD). Values were considered to be significant at $P < 0.05$.

Results

Microbial growth on watermelon slices from untreated fruit

As shown in Figure 1, aerobic bacteria on packaged watermelon slices grew at nearly a logarithmic rate during 12-d storage at 5 °C. Bacterial populations increased significantly from < 2 log cfu g^{-1} on the day of processing to > 7 log cfu g^{-1} after 12 d of storage. Lactic acid bacteria and yeasts and molds grew at lower rates during storage than aerobic bacteria. Populations of lactic acid bacteria and yeasts and molds did not increase significantly from day 0 to day 6. By day 12 both of these microbial groups displayed significant growth, increasing from < 2 to approximately 3 and 4 log cfu g^{-1} for lactic acid bacteria and for yeasts and molds, respectively.

Ethylene treatment of whole watermelons on juice leakage and microbial growth on subsequently processed and packaged slices

At the time of fresh-cut processing, populations of aerobic bacteria, lactic acid bacteria, and yeasts and molds from ethylene-treated fruit were not significantly different than those from control fruit (Figure 2). However, significantly higher populations of all microbes examined were noted on the ethylene-treated tissue after 6-d storage at 5 °C. After 12-d storage the populations of aerobic and lactic acid bacteria were significantly higher (by approximately 2 log units) in the slices from ethylene-treated fruit compared to the controls. No significant differences were noted in yeast and mold populations at day 12. The 10 ppm ethylene treatment induced excessive juice leakage from the watermelon slices while no leakage was noted in control slices (Table 1).

1-MCP treatment of whole watermelons on microbial growth of subsequently processed and packaged slices

Treatments of whole watermelons with 1-MCP alone did not affect microbial populations on subsequently processed and packaged slices compared to those on slices processed from control fruit (Figure 3). No significant differences were detected between the 0.5 ppm and 1.0 ppm 1-MCP treatments throughout the duration of the experiment.

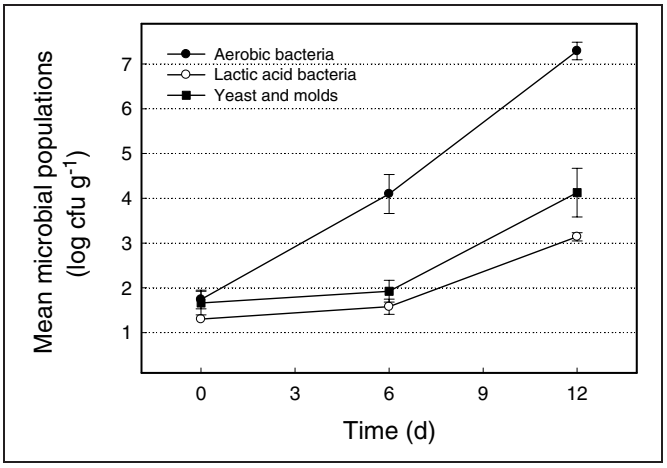

Figure 1—Microbial growth on packaged watermelon slices processed from control watermelons during storage at 5 °C. Error bars represent standard errors of the means.

Ethylene treatment without and with prior 1-MCP treatment of whole watermelons on juice leakage and microbial growth of subsequently processed and packaged slices

Microbial populations of aerobic bacteria, lactic acid bacteria, and yeasts and molds were smaller in ethylene-treated watermelon tissue that had been treated with 0.5 or 1.0 ppm 1-MCP compared to those treated with ethylene alone (Figure 4). On the day of processing, aerobic bacterial populations were significantly smaller (by about 2 logs) in the 1-MCP + ethylene-treated tissues compared to the ethylene-treated tissues, irrespective of 1-MCP concentration applied. Populations of yeasts and molds and lactic acid bacteria in slices from 1-MCP + ethylene-treated fruits were not significantly different from those in slices from ethylene-treated fruits on the day of processing. However, populations of all microbial groups examined were significantly smaller in the slices from 0.5 ppm 1-MCP-treated fruits after 6-d storage at 5 °C compared to those in slices from fruits treated with ethylene alone. After 12 d of storage significant differences among the treatments were seen with populations of aerobic bacteria and yeasts and molds but not lactic acid bacteria. Juice leakage normally induced by ethylene was prevented by either 0.5 or 1.0 ppm 1-MCP (Table 1).

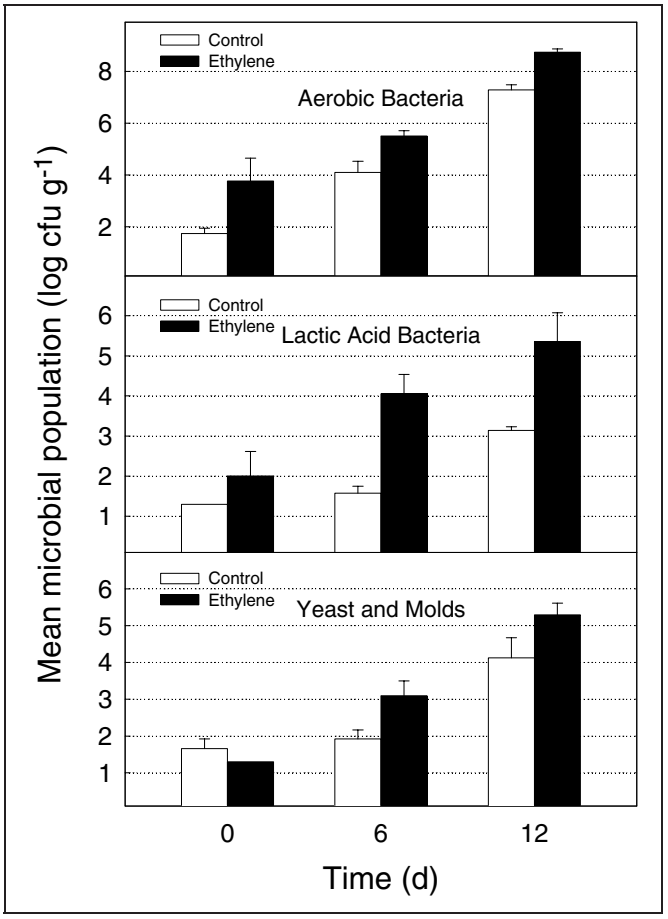

Figure 2—Effect of ethylene treatment of whole watermelons on microbial growth of subsequently processed and packaged watermelon slices stored at 5 °C. Untreated (control); treatment with 10 ppm ethylene for 5 d at 20 °C (ethylene). Error bars represent standard errors of the means.

Table 1—Juice leakage from packaged watermelon slices

Treatment[a]	Storage day	Juice leakage (mL)
Control	0	0
	6	0
	12	0
Ethylene	0	0
	6	6.33 ± 1.81
	12	12.18 ± 4.47
0.5 ppm 1-MCP + ethylene	0	0
	6	0
	12	0
1.0 ppm 1-MCP + ethylene	0	0
	6	0
	12	0

[a]Control = untreated; ethylene = treatment with 10 ppm ethylene for 5 d at 20 °C; 0.5 ppm 1-MCP + ethylene = treatment with 0.5 ppm 1-MCP for 18 h at 20 °C prior to treatment with 10 ppm ethylene; 1.0 ppm 1-MCP + ethylene = treatment with 1.0 ppm 1-MCP for 18 h at 20 °C prior to treatment with 10 ppm ethylene.

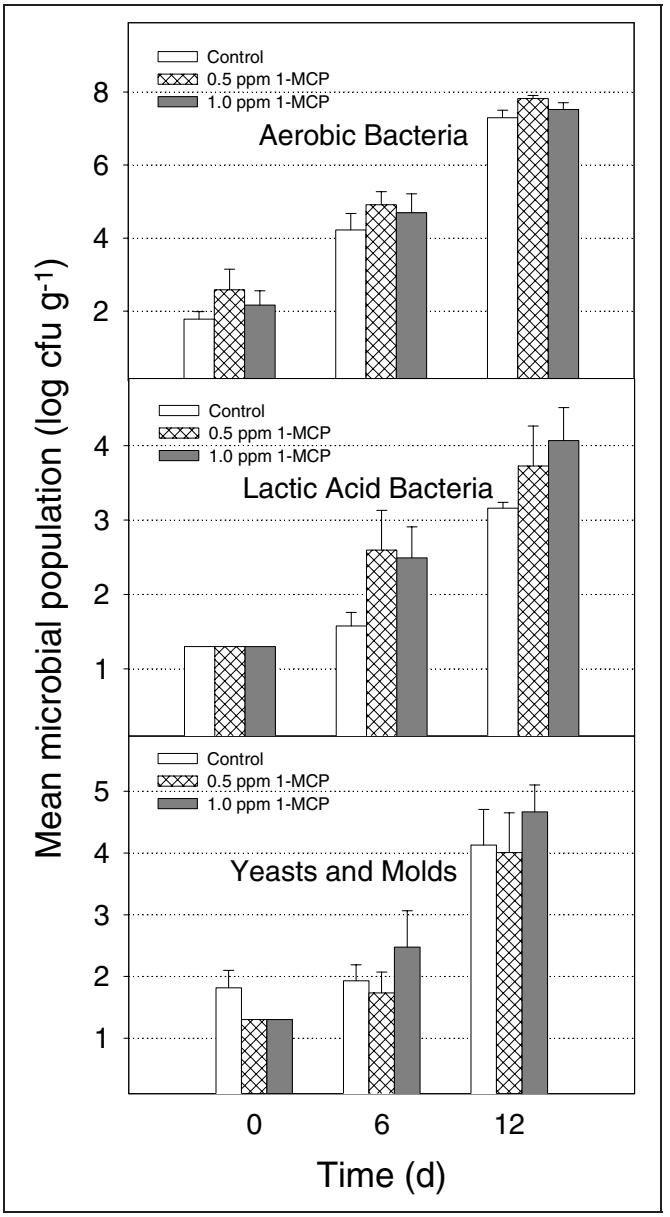

Figure 3—Effect of 1-MCP treatment of whole watermelons on initial microbial populations of subsequently processed and packaged watermelon slices. Untreated (control); treatment with 1-MCP (0.5 ppm 1-MCP or 1.0 ppm 1-MCP) for 18 h at 20 °C. Error bars represent standard errors of the means.

Time from harvest to 1-MCP treatment of whole watermelons on microbial populations of subsequently processed and packaged slices

At 6 and 12 d after processing, the populations of aerobic bacteria on watermelon slices prepared from fruit treated with 1-MCP 1 wk after harvest were significantly larger (by about 1 log) than the populations on slices from fruit treated 1 d after harvest (Figure 5). No significant differences, however, were noted in the lactic acid bacteria or the yeast and mold populations between the 2 treatments.

Ethylene treatment of whole watermelons on microbial populations within whole fruits

Treating whole watermelons with 10 ppm of ethylene resulted in significant aerobic bacterial growth (2.8 to 3.4 log cfu g^{-1}) throughout the tissues within 7 d of storage at 20 °C when compared to air-treated controls. Bacteria in the controls (approximately 3.7 log cfu g^{-1}) were only found within the first 10 mm of rind tissue and not beyond. The ethylene-treated tissues harbored approximately 3 log cfu g^{-1} to a depth of at least 100 mm.

Discussion

In the commercial produce industries, various fruits are often stored together during the transport and marketing period. Ethylene-induced effects need to be considered in such instances. During this period, nonclimacteric fruit such as watermelon may become exposed to ethylene being emitted from nearby climacteric fruit as well as from other sources such as the exhaust from diesel-powered forklifts. Exogenous ethylene can cause physiological disorders in watermelon fruit, inducing softening, water soaking, and other physiological characteristics (Mao and others 2006) that may stimulate microbial growth and fruit spoilage. As is evident from our results, ethylene induced both juice leakage and increased microbial growth in fresh-cut watermelon. Furthermore, ethylene exposure resulted in aerobic bacterial growth throughout the flesh of whole watermelon indicating that the microbes can breach the softened rind of the ethylene-exposed fruit. Our results also indicated that these negative effects of ethylene on watermelon quality can be avoided by treating fruit with low (0.5 or 1.0 ppm) concentrations of 1-MCP immediately after harvest and before the fruits are exposed to ethylene during transport and marketing. Mao and

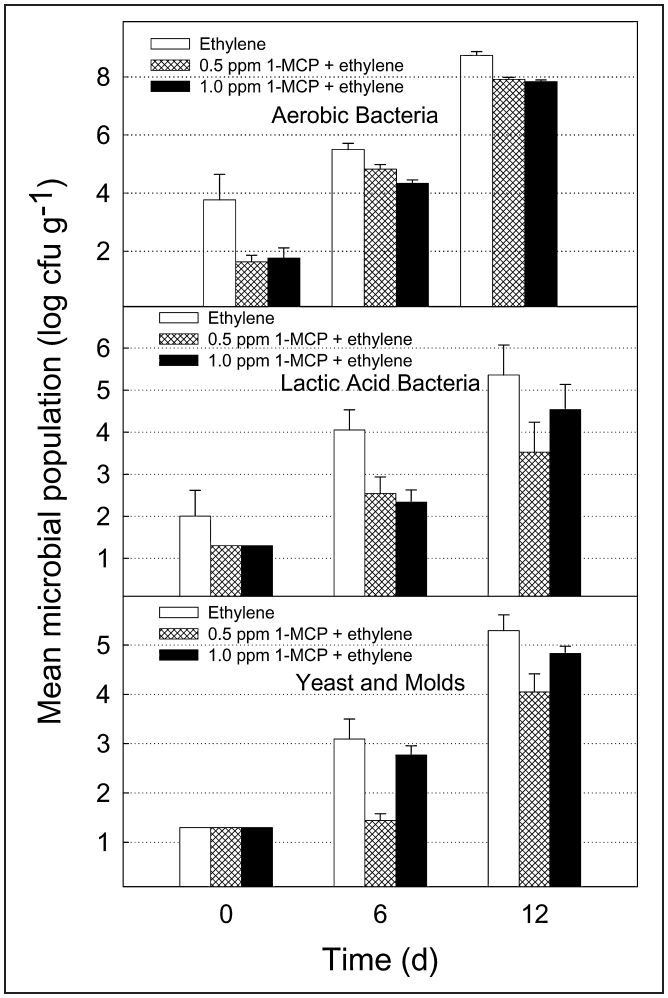

Figure 4—Effect of ethylene treatment without and with prior 1-MCP treatment of whole watermelon on microbial growth of subsequently processed and packages slices stored at 5 °C. Control treatment with 10 ppm ethylene for 5 d at 20 °C (ethylene); treatment with 0.5 ppm (0.5 ppm 1-MCP + ethylene) or 1.0 ppm (1.0 ppm 1-MCP + ethylene) of 1-MCP for 18 h at 20 °C prior to treatment with 10 ppm ethylene.

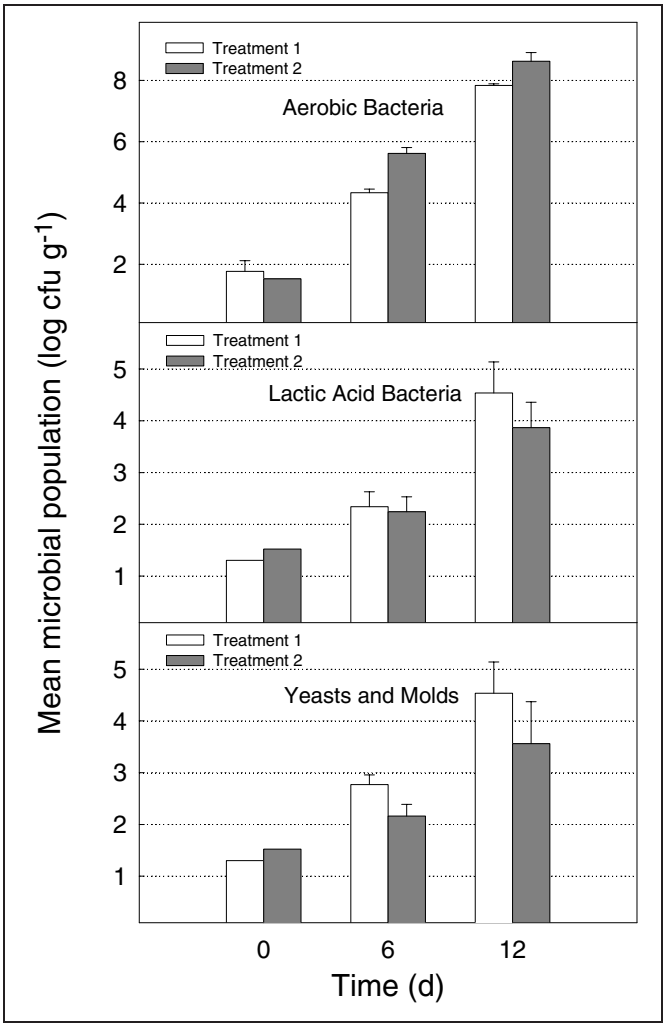

Figure 5—Effect of time period between harvest and 1-MCP treatment of whole watermelons on microbial populations of subsequently processed and packaged slices during storage at 5 °C. Treatment with 1-MCP 1 (Treatment 1) or 7 (Treatment 2) d after harvest. Error bars represent standard errors of the means.

others (2004) reported that exposure of watermelons to 10 ppm of 1-MCP for 18 h completely suppressed ethylene-induced softening, electrolyte leakage, and loss of extractable juice. Our findings indicated that even lower concentrations of 1-MCP are sufficient to avoid watermelon tissue damage and microbial growth associated with ethylene exposure. Treatment with 1-MCP will likely maintain quality and shelf stability of whole and fresh-cut watermelon.

In this study, we concluded that 1-MCP alone had no direct effect on the growth of microbes. However, 1-MCP did effectively counteract the ethylene-induced population increases of aerobic and lactic acid bacteria as well as yeasts and molds on watermelon slices. These results suggest that 1-MCP acts indirectly, likely by blocking ethylene action and the resultant physiological effects in watermelon tissues, to inhibit growth of a variety of microbes on watermelons. This wide spectrum of growth inhibition is preferred since the makeup of the microbial communities present on fresh-cut watermelon may be as important as the microbial populations in influencing the quality of the product over time.

Extending the time period from harvest to 1-MCP treatment resulted in moderately increased aerobic bacterial populations on subsequently processed watermelon slices while not significantly affecting lactic acid bacteria or yeast and mold populations. Thus the timing of 1-MCP treatment made little difference in the overall microbial populations as long as the treatment occurred prior to ethylene exposure.

Although there are no set US governmental limits on acceptable microbial populations on fresh-cut fruit such as watermelon, the growth and metabolism of microbes are key factors known to negatively affect the quality and shelf stability of fresh-cut produce.

Conclusion

Low dosage 1-MCP treatments of whole watermelon had no direct effect on microbial quality in subsequently processed watermelon slices. Treatment of whole watermelons with 1-MCP prior to ethylene exposure did effectively counteract ethylene-induced population increases of aerobic and lactic acid bacteria as well as yeasts and molds. Therefore, in a postharvest setting it may be beneficial to use low concentrations of 1-MCP as a quality assurance treatment to avoid the deleterious effects of possible ethylene exposure during storage or transport.

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