MAINTAINING POSTHARVEST QUALITY OF THE TOMATO FRUIT BY EMPLOYING METHYL JASMONATE AND ETHANOL VAPOR TREATMENT

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ABSTRACT

*The postharvest quality of tomato fruit (*Lycopersicon esculentum *L.) was evaluated after treatment with natural volatile compounds (methyl jasmonate [MJ] or ethanol) and storage at 13C during or following vapor exposure. The fruit treated with natural volatiles did not differ on fruit decay during vapor exposure, but following exposure and transfer to ambient air, the fruit had less decay at storage temperature. Volatile-treated fruit tended to maintain firmness during exposure, and the effects were significant for ethanol-treated fruit, following storage to ambient air. Sugar (i.e., fructose and glucose) concentration was stimulated in ethanol-treated fruit following exposure and transfer to ambient air. Ascorbic acid concentration was stimulated in MJ-treated fruit during exposure and persisted (including ethanol treatment) following transfer to ambient air. Total phenolics declined during vapor exposure and increased for MJ-treated fruit after transfer to ambient air. Lycopene concentration did not differ during MJ exposure but increased following volatile exposure. The fruit samples treated with vapors had accelerated percentage weight loss compared with untreated fruit during ripening but without commercial interest, whereas citric acid content did not differ among the treatments. The results suggest that MJ and ethanol vapor may improve fruit quality-related attributes on top of the well-documented antimicrobial protection during fresh produce storage and transit.*

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PRACTICAL APPLICATIONS

The data presented in this work suggest that the use of natural volatiles is an innovative and useful tool as an alternative to the use of synthetic fungicides or other sanitation techniques in storage/packaging. Vapor enrichment may reduce disease development with a major contribution to limiting the spread of the pathogen by lowering the spore load (spore production) in the storage/transit atmospheres as well as the use of natural volatiles as an alternative food preservative. In addition to these, the improvement of fruit quality observed in the present work is of great market importance. The effects of natural compounds on individual microorganisms (fungi and bacteria), both responsible for spoilage and foodborne pathogens, as well as the minimum concentration to gain effectiveness without affecting fresh produce quality and storage deserve further research.

INTRODUCTION

The control of postharvest decay of fruits and vegetables is typically achieved through sound cultural practices, fungicide applications, controlled temperature and storage environments. In recent years, as the use of synthetic fungicides is becoming increasingly limited and regulated, and consumers demand agricultural commodities without pesticide residues, new preservation technologies are needed, which have to be considered as human-safe and environmentally friendly. Among the various alternatives, there is an increasing interest in the possible use of natural compounds, which are biodegradable and eco-friendly, to prevent microbial growth in the food items, thus answering to consumer's pressure to reduce chemical additives in foods and are less damaging to the human health and environment, and for pest and disease control in agriculture (Duru *et al.* 2003).

Natural volatile compounds, including plant growth regulators such as methyl jasmonate (MJ), have been extensively investigated and demonstrated to elicit defense responses from the plant (Yao and Tian 2005; Gonzalez-Aguilar *et al.* 2006) as well as antibacterial and antifungal activities (Moline *et al.* 1997; Droby *et al.* 1999). MJ treatments regulate diverse processes such as skin color development (by promoting β-carotene synthesis and chlorophyll degradation), ion leakage and considerable benefits associated with reduced chilling injury symptoms (reviewed by Gonzalez-Aguilar *et al.* 2006).

Ethanol is a common food component, which is produced in many plants, and an inexpensive and well-studied natural substance present in many food products that can easily be metabolized in many plants and mammals with potent antimicrobial activity (Larson and Morton 1991). The use of ethanol

should be less than that of synthetic fungicides. It should pose a minimal ingestion hazard to humans because of its low mammalian toxicity (Karabulut *et al.* 2004). Ethanol dips and vapors were reported to control postharvest diseases of peaches, table grapes and apples (Larson and Morton 1991; Feliciano *et al.* 1992; Ghahramani *et al.* 2000; Mlikota Gabler and Smilanick 2001).

Fruit quality encompasses many aspects and includes not only flavor, color, nutritional aspects and firmness, but also shelf life, processing attributes and resistance to pathogens (Brummell and Harpster 2001). The effect of natural volatiles on postharvest decay and quality was investigated on only a few fresh commodities (i.e., papaya [Gonzalez-Aguilar *et al.* 2003]; pears [Ju *et al.* 2000]; kiwifruit [Wang and Buta 2003]; and tomato and strawberry [Tzortzakis 2007a]). Moreover, previous works have shown that ethanol and acetaldehyde can retard ripening in some fruits, such as tomatoes (Kelly and Saltveit 1988; Beaulieu *et al.* 1997; Yanuriati *et al.* 1999), while it can stimulate ripening in others, such as kiwifruit (Mencarelli and Savarese 1991). The present study was undertaken to determine if these natural products would reduce decay and maintain a better quality and shelf life of tomato fruit.

MATERIALS AND METHODS

Plant Materials

Tomato (*Lycopersicon esculentum* L. cv. Carousel) fruits obtained from a local market were selected for uniformity in size, appearance, ripeness and the absence of physical defects. The selected fruits were randomized before being used for treatments with various volatiles.

Treatments with Natural Volatiles

Six fruits for each individual treatment were placed into 0.95-L polystyrene containers with snap-on lids. Volatile compounds (Sigma Aldrich, Athens, Greece) used in this study included MJ (44.8 μL/L) and absolute ethyl alcohol (AEA, 1.2 mL/L) diluted in dH_2O. Aliquot (30 mL) of each volatile solution was placed into individual small beakers, which were subsequently placed inside the plastic containers just before the lids were covered. Filter paper moistened with water was placed into each container, maintaining a high relative humidity (RH ~95%) during the storage period. The volatile compounds were allowed to vaporize inside the containers spontaneously at 20C for 8 h. The containers were then transferred to storage at 13C in a cold room. Tomato fruits were exposed to the control (ambient air) or volatiles for 1 week

at 13C. Following exposure, a second batch of fruits were transferred to ambient air and stored at 13C for an additional 1 week. The control samples were handled similarly with the exception of the volatile treatment. All experiments were repeated twice.

Decay Evaluation

The severity of decay was visually evaluated during and following exposure to volatile compounds at 13C/95% RH. The degree of infection on the fruit was rated using a scale of 1–5, where 1 = clean, no infection; 2 = trace infection; 3 = slight infection; 4 = moderate infection; and 5 = severe infection.

Quality Analysis

The fruits ($n = 6$) were labeled and the weight was recorded prior to exposure to ambient air or volatile-enriched air (MJ or AEA) at 13C and 95% RH. The fruits were weighed after a 1- or 2-week intervals, and the percent weight loss of original weight was computed. Fruit firmness was measured at two points on the shoulder of the tomato fruit ($n = 6$), respectively, for each treatment by applying a plunger of 8 mm in diameter, using a penetometer FT 011 (TR Scientific Instruments, Forli, Italy). The amount of force (kg/cm^2) required to compress the radial pericarp (i.e., surface) of each tomato was recorded at ambient temperature. Soluble sugars were extracted from the tomato ($n = 4$) tissue (*ca.* 0.5 g) in 80% (v/v) methanol. Extracts were passed through ion exchange columns (Dowex AG50W and Amberlite IRA-67 in series, Sigma Aldrich, Poole, UK). Soluble sugar composition was analyzed via high-performance liquid chromatography (HPLC) and pulsed amperometric detection (ED40 electrochemical detector, Dionex, Sunnyvale, CA). Sample components were eluted from the column isocratically using 150 mM NaOH at a flow rate of 1 mL/min (Adams *et al.* 1992). Peak area was recorded and results expressed as nanomole sugars per gram fresh weight. Titratable acidity (TA) was determined by potentiometric titration, using fruit samples (5 g) diluted in 100-mL distilled water and titrated with 0.1 N NaOH with the formation of a pink precipitate and monitored using phenolphthalein as pH indicator. The reported values ($n = 6$) were expressed in terms of citric acid percentage. Total phenolic concentration was determined from blended fruit ($n = 4$) tissue extracts (5 g) of following repeated (fourfold) addition of 2.5 mL of 50% (v/v) methanol under ultrasonication. Aliquots were collected and centrifuged for 5 min at 4C at 3,000 × g. The supernatant was transferred to a fresh Eppendorf tube (Sigma Aldrich) and 125 µL was pipetted into a fresh test tube, to which 1.5 mL of water (Milli-Q), 125 µL of Folin–Ciocalteu's reagent (Sigma Aldrich) and 1.25 mL of 7% (w/v) sodium carbonate were added. The reaction

mix (3 mL) was incubated in the dark for 1.5 h, prior to reading the absorbance at 755 nm (Genesys 10 Vis, ThermoSpectronic, Rochester, NY). The results were expressed in terms of gallic acid equivalents (Sigma Aldrich) per 100-g fresh weight of tissue. Ascorbic acid (consisting the major part in vitamin C) in tomato juice ($n = 4$) was determined by the 2,6-dichloroindophenol titrimetric method (Helrich 1990).

Carotenoid (lycopene and β-carotene) composition was determined from homogenized tomato ($n = 4$) tissue (*ca.* 5 g), which was added to a round-bottomed flask with 4-g silica gel and subjected to rotary evaporation under vacuum at a maximum of 35C. Methanol (10 mL) was added to capture the remaining water and assist in the transfer of lipophilic carotenoids, prior to ultrasonication and evaporation under vacuum. Volumes of 10 mL tetrahydrofuran and 10 mL of acetone were added, and the solution was subjected to ultrasonication. This procedure was repeated three times, until a colorless tomato tissue was obtained. The extracts were bulked together in a conical flask, then subjected to evaporation under vacuum prior to resuspension in *ca.* 40 mL ethyl acetate and dH_2O. Employing a separation funnel, the organic phase (colored layer) was collected and dried, using anhydrous Na_2SO_4, prior to evaporating the solvent under vacuum in a fresh flask. The residue was reextracted in 10 mL ethyl acetate, and stored at –20C prior to analysis. Standard precautions were taken throughout to prevent the exposure of carotenoids to light, oxygen, acid and heat. The carotenoids were determined by means of HPLC analysis on an RP18 Lichrospher 100 (Merck, Darmstadt, Germany) 250×4 (5 μ) column and DAD detection. Eluent (A) was acetonitrile : water (9:1) containing 0.1% triethylamine and (B) ethyl acetate, and the flow rate was 1 mL/min. The elution program was used as follows: from 100% A to 0% A in 25 min (total run time 35 min). The temperature of the column was kept at 40C and monitoring was performed at 450 nm (β-carotene) and 471 nm (lycopene). Calibration curves were prepared at a range of 0–186 nmol/mL lycopene and β-carotene, employing HPLC gradient standards.

Statistical Analysis

Data were first tested for normality, and then subjected to analysis of variance (ANOVA). Sources of variation were time of storage and treatments. Significant differences between mean values were determined using Duncan's multiple range test ($P = 0.05$) following one-way ANOVA. Significant differences on percentage values (weight loss) were logarithmic transformed prior to using ANOVA. Statistical analyses were performed using SPSS (SPSS Inc., Chicago, IL), and graphs were produced using Prism v.2.0 (Graph Pad Inc., San Diego, CA).

RESULTS AND DISCUSSION

Effect of Volatile Vapor on Fruit Decay

Vapor-treated fruit maintained ($P < 0.05$) severity of decay (mainly anthracnose rot (*Colletotrichum coccodes*) and secondary black spot (*Alternaria alternata*)) following vapor exposure and transfer to ambient air, with MJ showing the least fruit decay, whereas nontreated fruit stored in ambient air had increased fruit deterioration (Fig. 1). *In vivo* and *in vitro* vapor studies with *C. coccodes* did not affect the mycelium growth, whereas *in vivo* studies retarded the shift of the fungus from vegetative to reproductive phase, implying that the suppression of pathogen development was because, in a large part, of the impacts of volatiles on fruit–pathogen interactions on fruit tissue, with MJ acting as the plant defense elicitor (Tzortzakis 2007b). In previous reports, volatile compounds reduced fruit decay during postharvest treatments in several fresh produce including raspberry, kiwi, strawberry and tomato fruit (Wang 2003; Wang and Buta 2003; Tzortzakis 2007a).

Effect of Volatile Vapor on Fruit Quality

The percentage weight loss was greater ($P < 0.05$) for volatile-treated fruit during and/or following exposure to volatiles compared with fruit maintained in

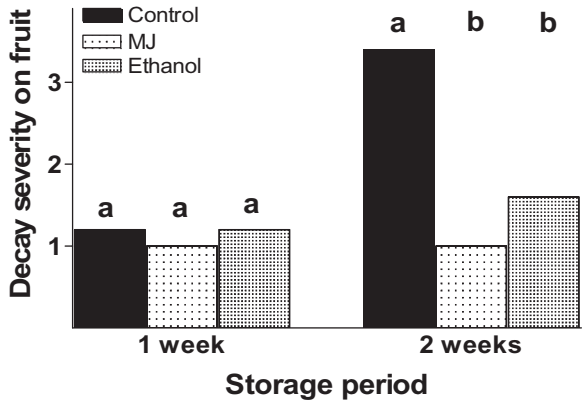

FIG. 1. EFFECTS OF METHYL JASMONATE (MJ) (44.8 µL/L) OR ETHANOL (1.2 mL/L) VAPOR ON THE SEVERITY OF DECAY IN TOMATO FRUIT DURING (1 WEEK) OR FOLLOWING (2 WEEKS) EXPOSURE TO VAPOR AT 13C/95% RELATIVE HUMIDITY
Scoring (mean value) represents a visual rating of decay severity on fruit using a scale of 1–5 with 1 = clean, no infection; 2 = trace; 3 = slight; 4 = moderate; and 5 = severe infection. Values (n = six replicates per treatment) followed by the same letter do not differ significantly at $P = 0.05$ according to Duncan's multiple range test.

ambient air (Table 1). Storage period and treatment, but not their interactions, had a significant ($P < 0.05$) effect on fruit weight loss. However, fruit weight loss was minute for all the treatments (below 2%). Previous experiments using eugenol, thymol or menthol vapors revealed benefits because of the reduced weight loss in cherries and grapes (Martinez-Romero *et al.* 2005; Serrano *et al.* 2005). Moreover, strawberry and tomato fruit exposed to essential oil vapors were not differentiated on weight loss basis (Tzortzakis 2007a).

Fruit firmness maintained up to 40% ($P < 0.05$) in fruit previously exposed to ethanol (1.2 mL/L) for 1 week compared with fruit subjected to traditional storage conditions throughout, whereas the effect did not persist following fruit transfer/storage for 1 week in ambient air (Table 1). MJ enrichment resulted in no change in fruit firmness during or following volatile exposure, contrasting previous reports with MJ-treated fresh-cut pineapple (Martinez-Ferrer and Harper 2005). However, the exposure of papaya (*Carica papaya* L. cv. Sunrise) fruit to MJ vapors for 16 h at 20C resulted in reduced firmness during storage and shelf life because of rapid fruit deterioration at 20C (Gonzalez-Aguilar *et al.* 2003). Several studies highlighted the beneficial effects of natural volatiles, including essential oils, on fruit firmness by fruit ripening inhibition (Pesis *et al.* 1998; Martinez-Romero *et al.* 2005; Tzortzakis 2007a).

TA of treated fruit did not differ during vapor exposure or following storage in ambient air (see Table 1), being in accordance with previous reports on essential oil-treated fruit (Tzortzakis 2007a). Impacts of ethanol vapor resulted in increased levels of soluble sugars (fructose and glucose; i.e., the predominant soluble carbohydrates fractions), with significant ($P < 0.05$) effects when ethanol-treated fruits were subsequently transferred to ambient air (Table 2) that may have reflected the weight losses observed during exposure. However, no differences were observed in MJ-treated fruit, contrasting

TABLE 1.
EFFECTS OF METHYL JASMONATE (MJ) 44.8 μL/L) OR ETHANOL (1.2 mL/L) VAPOR ENRICHMENT ON WEIGHT LOSS, FRUIT FIRMNESS AND TITRATABLE ACIDITY IN TOMATO FRUIT AFTER 1-WEEK EXPOSURE OR FOLLOWING ITS TRANSFER TO AMBIENT AIR FOR AN ADDITIONAL 1 WEEK

	Weight loss (%)			Firmness (kg/cm^2)			Titratable acidity (% citric acid)		
	0	1 week	2 weeks	0	1 week	2 weeks	0	1 week	2 weeks
Control	0	0.22^b	1.56^a	3.12	2.11^b	2.40^a	5.16	3.81^a	4.33^a
MJ		0.48^a	1.91^a		2.54^{ab}	2.34^a		3.96^a	4.01^a
Ethanol		0.42^a	1.67^a		2.93^a	2.11^a		4.36^a	3.96^a

The fruits were maintained throughout at 13C and 95% relative humidity. Values represent the mean of measurements made on four independent fruit per treatment. Values followed by the same letter in each column do not differ significantly at $P = 0.05$ according to Duncan's multiple range test.

the report on fresh-cut kiwifruit slices (Wang and Buta 2003). Moreover, MJ-treated papaya retained higher organic acids than the control fruit (Gonzalez-Aguilar *et al.* 2003), whereas MJ- and tea tree oil-treated raspberries maintained higher levels of sugars and organic acids compared with untreated fruit (Wang 2003). Basil oil spray emulsion (0.16% v/v) treatment affected the texture and the flavor of the banana fruit but did not have any significant effect on total soluble solids (Anthony *et al.* 2003).

Ascorbic acid concentration in tomato fruit tended to increase throughout fruit storage during exposure to MJ or ethanol vapors (Table 3). The effect was significantly different ($P < 0.05$) for MJ-treated fruit during or following exposure, whereas that effect was obtained in ethanol-treated fruit only after the exposure and storage to ambient air for 1 week. Vitamin C (ascorbic and L-dehydroascorbic acid) has a high antioxidant power, providing protection against the presence of free radicals and consequently participating in the prevention of many degenerative diseases as well as it is an essential nutrient for human.

MJ-treated fruit stimulated (increased tendency) lycopene concentration during exposure and significant effects ($P < 0.05$) were observed following exposure and transfer to ambient air (Table 3). Indeed, ethanol vapors did not affect the lycopene concentration, contrasting previous reports whereas ethanol application inhibited tomato fruit ripening as a result of elevated endogenous levels of acetaldehyde in the fruit (Beaulieu *et al.* 1997). However, no differences revealed for β-carotene concentration among the treatments during or following volatile exposure.

Vapor treatments tended to decrease total phenolic concentration of fruit during vapor exposure with significant differences in ethanol-treated fruit

TABLE 2.
EFFECTS OF METHYL JASMONATE (MJ) (44.8 µL/L) OR ETHANOL (1.2 mL/L) VAPOR ENRICHMENT ON GLUCOSE, FRUCTOSE AND TOTAL SUGAR CONCENTRATION IN TOMATO FRUIT AFTER 1-WEEK EXPOSURE OR FOLLOWING ITS TRANSFER TO AMBIENT AIR FOR AN ADDITIONAL 1 WEEK

	Glucose (nanomole per gram fresh weight)			Fructose (nanomole per gram fresh weight)			Total sugars (nanomole per gram fresh weight)		
	0	1 week	2 weeks	0	1 week	2 weeks	0	1 week	2 weeks
Control	0.211	0.322[a]	0.167[b]	0.146	0.225[a]	0.116[b]	0.364	0.555[a]	0.288[b]
MJ		0.307[a]	0.137[b]		0.220[a]	0.093[b]		0.534[a]	0.236[b]
Ethanol		0.336[a]	0.378[a]		0.234[a]	0.263[a]		0.578[a]	0.663[a]

The fruits were maintained throughout at 13C and 95% relative humidity. Values represent the mean of measurements made on four independent fruit per treatment. Values followed by the same letter in each column do not differ significantly at $P = 0.05$ according to Duncan's multiple range test.

TABLE 3.
EFFECTS OF METHYL JASMONATE (MJ) (44.8 μL/L) OR ETHANOL (1.2 mL/L) VAPOR ENRICHMENT ON ASCORBATE, TOTAL PHENOLIC, LYCOPENE AND β-CAROTENE CONCENTRATION IN TOMATO FRUIT AFTER 1-WEEK EXPOSURE OR FOLLOWING ITS TRANSFER TO AMBIENT AIR FOR AN ADDITIONAL 1WEEK

	Ascorbate (milligram per gram fresh weight)			Phenolics (micromole per gram fresh weight)			Lycopene (milligram per gram fresh weight)			β-Carotene (milligram per gram fresh weight)		
	0	1 week	2 weeks	0	1 week	2 weeks	0	1 week	2 weeks	0	1 week	2 weeks
Control	0.09	0.08[b]	0.06[b]	2.06	2.12[a]	1.83[b]	298	156[a]	221[b]	30.7	23.6[a]	31.9[a]
MJ		0.13[a]	0.09[a]		1.86[a]	3.43[a]		267[a]	614[a]		20.2[a]	45.7[a]
Ethanol		0.12[ab]	0.08[a]		1.50[b]	2.18[b]		437[a]	427[ab]		34.7[a]	46.9[a]

The fruits were maintained throughout at 13C and 95% relative humidity. Values represent the mean of measurements made on six independent fruit per treatment. Values followed by the same letter in each column do not differ significantly at $P = 0.05$ according to Duncan's multiple range test.

(Table 3). However, phenolic concentration was stimulated (increased tendency) following exposure and transfer to ambient air, with significant effects in MJ-treated fruit. A similar pattern was observed in essential oil-treated tomato and strawberry fruit (Tzortzakis 2007a). Most of the antimicrobial activity in essential oils from spices and culinary herbs appears to be associated with interactions between phenolic compounds and the food matrix (Nuchas and Tassou 2000).

Interestingly, volatile treatment resulted in a significant change in the antioxidant properties (ascorbate and lycopene concentration) exhibited by the tomato fruit. Impacts of storage treatments on the antioxidative properties and products of tomato are of concern because tomato fruits are recognized to be particularly rich in several antioxidants such as vitamin C, carotenoids (especially lycopene), vitamin A plus some flavonoids and other phenolic compounds, and they protect the human body tissue against oxidative attacks (Abushita *et al.* 1997; Scalfi *et al.* 2000).

Phenolic compounds are plants' secondary metabolites, which have been reported to be important determinants in both sensory and nutritional quality of fruits and vegetables (Tomas-Barberan and Espin 2001). The daily intake of phenolic compounds is about 1 g (Scalbert and Williamson 2000). Particularly, phenols contribute substantially to the antioxidant complement of many fruit species, by playing an important role in inhibiting reactions mediated by reactive oxygen species, which are associated with a number of human diseases (Karakaya and Tas 2001) that have potential health effects (Heinonen *et al.* 1998). In addition, epidemiologic studies showed that high fruit and vegetable consumption has health benefits in the prevention of chronic diseases such as cardiovascular disease and cancer (Arai *et al.* 2000). Previous studies suggested that the increase of the phenolic compounds resulted in the increase of the antioxidant activity in sweet basil by MJ treatment, which in consequence, stimulate plant defense mechanisms (Kim *et al.* 2006). In this regard, it is worth noting the marked stimulation in ascorbic acid and lycopene concentration for MJ-treated fruit that might have increased the phenolic content. However, in the present study, the respiration rates were not examined and the potential of anaerobic condition need to be evaluated in detail in the future. The possible anaerobic condition to some extent, in the present study, may have contributed to slowing down the ripening process, maintaining fruit firmness as well as enhancing induced resistance of the fruit. The minimum concentration of oxygen for most fruit is around 1.5%, although various fruits may require high levels or can tolerate brief exposure to lower levels. Generally, approximately below 1.2% oxygen, anaerobic respiration takes place, resulting in the production of alcohols, lactic acid or acetaldehyde. These give the fruit objectionable flavor and aroma, and the fruit has a very much shortened life.

At the market interface, only produce that corresponds to the expectations of the consumer can survive. Fruit firmness is an important quality attribute and is directly related to the enhancement of the storability potential and the induction of greater resistance to decay and mechanical damage (Barret and Gonzalez 1994). It is known that the cell wall matrices, especially pectins, undergo disruption during fruit ripening, which results to fruit softening that accompanies ripening (Tucker and Greison 1987).

The present study highlights the potential for using natural volatiles for postharvest disease control and maintenance of fruit quality in storage and/or transit. Natural volatiles are known to be effective against a wide spectrum of microorganisms and leave no detectable residues. Indeed, these compounds may improve the fruit quality and storage of fresh produce. These findings may have considerable commercial significance, but first, efficacy must be proven by employing microbial studies (both *in vitro* and *in vivo*) and in a commercial context where produce is submitted to vapor enrichment in the usual storage/transit bins, cartons or boxes, including coating fruit with plant volatiles and/or modified atmosphere packaging on a wider range of fresh produce. Natural volatiles are not as broad spectrum as synthetic pesticides, but their effectiveness can be improved by using them in conjunction with carefully designed packaging. Considerable care is needed in the application of the natural volatiles as a continuous fumigant effect may result in the tainting of the product as thin-skinned products are more prone to tainting than those with thicker skins.

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