METHYL JASMONATE IMPROVES QUALITY OF STORED ZUCCHINI SQUASH

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ABSTRACT

*Freshly harvested zucchini squash (*Cucurbita pepo *L., cv. Elite) were pressure infiltrated (82.7 kPa for 3 min) with an aqueous suspension solution of 0.1 mM methyl jasmonate, while control fruit were similarly infiltrated with distilled water. All fruit were then stored at a chilling temperature of 5C. Chilling injury symptoms developed in the control fruit after 4 days of storage. However, the onset of chilling injury was significantly delayed by methyl jasmonate treatment. The methyl jasmonate treatment not only retarded the development of chilling symptoms in zucchini squash, but also maintained higher levels of carbohydrates and organic acids during storage compared with the controls. Fructose and glucose were major carbohydrates, and malic acid was the predominant organic acid in zucchini squash. Methyl jasmonate reduced the losses of these sugars and organic acids, and thus helped maintain better internal quality of zucchini squash during cold storage.*

INTRODUCTION

Jasmonic acid is a naturally occurring compound widely distributed in plants. Its methyl ester, methyl jasmonate, was first detected as a fragrant component of the essential oil of *Jasminum* and other plant species (Crabalona 1967). Several studies have shown that methyl jasmonate can inhibit growth and germination, promote respiration and senescence, and act as a growth regulator (Sembdner and Parthier 1993). Some of the physiological effects of methyl jasmonate are similar to those of abscisic acid, such as induction of stomatal closure and promotion of leaf abscission (Parthier *et al.* 1992). Methyl

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jasmonate was also found to stimulate ethylene production, increase ethylene-forming enzyme activity, and enhance fruit ripening (Saniewski *et al.* 1988).

In a previous study (Wang and Buta 1994), we found that application of methyl jasmonate delayed the onset of chilling injury in zucchini squash stored at low temperatures. The reduction of chilling injury in jasmonate-treated squash was accompanied by higher levels of abscisic acid and polyamines (Wang and Buta 1994). Increases in putrescine and spermidine contents by methyl jasmonate treatment have also been found in rice seedlings (Lee *et al.* 1996). Reduction of stomata opening and prevention of water loss were also induced by exogenous application of methyl jasmonate in rice seedlings. All of these responses may have contributed to the greater survival rate of treated rice seedlings at chilling temperatures. Increase in chilling tolerance by methyl jasmonate treatment has also been obtained in avocados, grapefruit, and bell peppers (Meir *et al.* 1996). Even though evidence has shown that methyl jasmonate can alleviate chilling injury, its effect on storage quality of chilling-susceptible commodities has not been investigated.

This study was undertaken to determine the influence of methyl jasmonate treatment on both external injury symptoms and internal quality and chemical composition including carbohydrate and organic acid contents in zucchini squash stored at a chilling temperature.

MATERIALS AND METHODS

Zucchini squash (*Cucurbita pepo* L., cv. Elite) at commercial maturity (18-22 cm in length) were harvested from a local farm near Beltsville, MD. Fruit free from blemishes were selected for this study. Samples were randomly divided into two lots and treated with 0 and 0.1 mM methyl jasmonate aqueous suspensions, respectively. Preliminary experiments using various concentrations of methyl jasmonate treatment have indicated that 0.1 mM was promising in alleviating chilling injury. Fruit were completely immersed in the solution for 3 min and an air pressure of 82.7 kPa was applied for the infiltration of methyl jasmonate into the tissue. Four to five grams of 0.1mM methyl jasmonate were infused into each squash by this method. Sixty fruit were used in each treatment. Following infiltration treatment, the fruit were placed on Kraft paper and allowed to air dry before storage at 5C. Three fruit from each treatment were transferred to 20C at 2-day intervals and chilling injury was evaluated after one day at 20C. Three two-gram flesh samples were taken from each fruit and used for the analysis of organic acids and carbohydrates.

The severity of chilling injury was judged subjectively based on the extent of surface pitting, water soaked areas, and decay. The degree of injury was expressed according to a numerical scale from 1 to 5, with 1 = no abnormality, 2 = trace, 3 = slight, 4 = moderate, and 5 = severe chilling injury. Squash with a degree of injury <3 were considered marketable.

For analysis of organic acids and carbohydrates, two grams of flesh tissue of zucchini squash were homogenized with a Polytron homogenizer (Brinkmann Instruments, Westbury, NY) in imidazole buffer (20 mM, pH 7.0). The extracts were centrifuged and the supernatants were dried *in vacuo* in derivatizing vials. Procedures described by Li and Schuhmann (1980) were modified for the derivatization of sugars. A known amount of β-phenyl-D-glucopyranoside was included in all samples as an internal standard. One mL Trisil reagent (Pierce, Rockford, IL) was mixed with each sample vigorously and then heated at 75C for 30 min. After silylation, one μL of each derivatized sample was injected into a Hewlett Packard 5890 gas chromatograph (Hewlett Packard, Palo Alto, Ca.) equipped with a flame ionization detector and a 25 m crosslinked methyl silicon gum capillary column (0.2 mm ID, 0.33 μm film thickness). Temperatures were as follows: injector 250C, detector 275C, and column 100 to 250C programmed at 10C/min with 0 min initial and 23 min final times. Organic acids were analyzed after extraction with imidazole buffer and purification with a Baker-10 solid phase extraction system. Supernatants from the extracts were passed through quaternary amine columns, which were previously conditioned with hexane and methanol. The samples were then eluted from the columns with 0.1 N HCl. The eluates were concentrated to dryness *in vacuo* in derivatization vials. Procedures of derivatization and chromatography for organic acids were the same as those for sugars except that column temperature was programmed from 180 to 250C at 10C/min with 3 min initial and 12 min final times. The sugars and organic acids were quantified by comparison with derivatized standards. A Hewlett Packard ChemStation was used to calibrate the peaks, record the data, and calculate the results.

RESULTS AND DISCUSSION

Chilling injury symptoms in zucchini squash first appear as surface pitting, followed by large sunken spots, water soaked areas, shrivelling, and decay. The symptoms of chilling injury usually become more pronounced after transfer of the squash from a chilling temperature to a warmer temperature. Zucchini squash in this study were evaluated for the severity of appearance of chilling injury one day following transfer from 5C to 20C. The difference in the development of chilling injury symptoms between methyl jasmonate treated and untreated zucchini squash is shown in Fig. 1. All squash appeared normal after two days of exposure to 5C. Traces of chilling injury were found on the skin of squash in the control group on the fourth day. Chilling injury symptoms became more apparent in these squash after four days of storage at 5C and reached the severe stage by the 12th day (severity of chilling injury at 5). However, methyl jasmonate treatment significantly delayed the onset of chilling injury. The treated squash did not develop surface pitting until after eight days of storage at 5C and were still in marketable conditions (severity of chilling injury at <3) after 12 days.

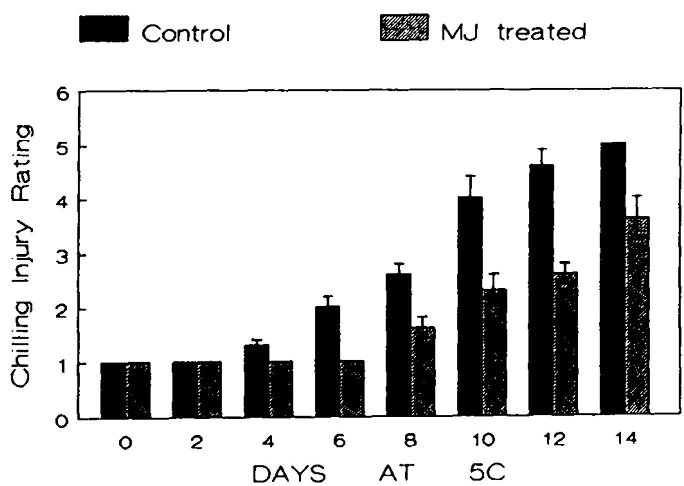

FIG. 1. EFFECT OF 0.1 mM METHYL JASMONATE (MJ) ON THE DEVELOPMENT OF CHILLING INJURY OF ZUCCHINI SQUASH STORED AT 5C
Chilling injury was rated as 1 = no abnormality, 2 = trace, 3 = slight, 4 = moderate, and 5 = severe pitting. Vertical bars represent SE with n = 3.

Alleviation of chilling injury by methyl jasmonate treatments has also been reported in avocados, grapefruit, and bell peppers (Meir *et al.* 1996) and in rice seedlings (Lee *et al.* 1996). Very low concentrations (2.5 μM) were needed to produce a favorable response in 'Fuerte' and 'Hass' avocados. In 'Etinger' avocados, 70% of control fruit had moderate to severe chilling injury while 70% of fruit treated with 10 μM methyl jasmonate had no or slight exocarp injury. It was postulated that jasmonates may play an integral role in the signal transduction cascade and replace other stress treatments that induce biochemical changes involved in enhanced resistance to chilling injury (Meir *et al.* 1996). Along with other stress-induced responses, increase in chilling tolerance of the jasmonate-treated rice seedlings was accompanied by an increase in putrescine and spermine levels. The accumulation of putrescine was suggested to be one of the factors required for inducing chilling tolerance in rice seedlings (Lee 1997). These observations are consistent with our findings that methyl jasmonate reduces chilling injury in zucchini squash in part through its regulation of polyamine levels (Wang and Buta 1994).

In other chilling-sensitive fruit, vegetable such as cucumbers, techniques which reduce chilling injury also improve storage quality including maintenance of sugar and organic acid levels (Wang and Qi 1997). Injury induced by low temperature exposure accelerates the deterioration of chilling- sensitive fruits and vegetables. Consequently, methods that increase the tolerance of these commodities to chilling exposure also retard degenerative processes. The losses of fructose (Fig. 2) and glucose (Fig. 3) in zucchini squash during storage at 5C were reduced by methyl jasmonate treatment. The greater retention of these sugars might not be a direct effect of methyl jasmonate treatment, but rather the result of less chilling stress.

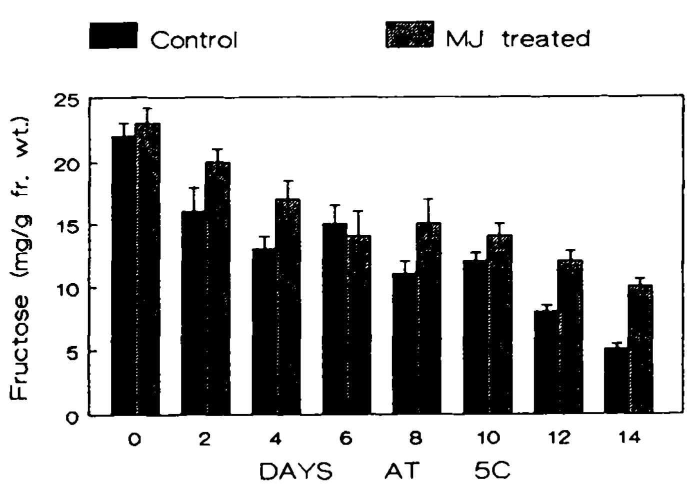

FIG. 2. EFFECT OF 0.1 mM METHYL JASMONATE (MJ) ON CHANGES OF FRUCTOSE CONTENT IN ZUCCHINI SQUASH DURING STORAGE AT 5C
Vertical bars represent SE with n = 3.

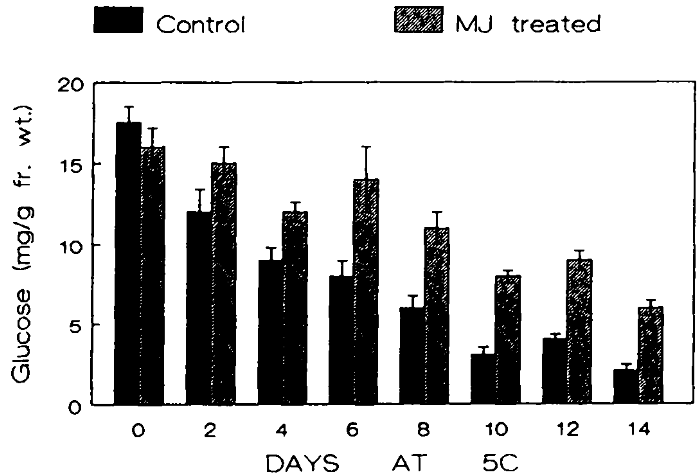

FIG. 3. EFFECT OF 0.1 mM METHYL JASMONATE (MJ) ON CHANGES OF GLUCOSE CONTENT IN ZUCCHINI SQUASH DURING STORAGE AT 5C
Vertical bars represent SE with n = 3.

Malic acid was the predominant organic acid in zucchini squash (Fig 4). Citric acid was also detected, but was present in much lower amounts (data not shown). There was a steady decrease in malic acid levels after the second day of storage at 5C in both methyl jasmonate-treated and control fruit (Fig. 4). However, a higher level of malic acid was maintained in treated squash than in control samples. A significant difference in malic acid levels between treated and control squash was detected after the fourth day of storage. The occurrence of the rapid decline of malic acid in control squash corresponded to the appearance of chilling injury symptoms. Therefore, the loss in malic acid might have been a result of chilling injury associated with temperature stress-related degenerative process.

Our study showed that chilling temperature not only caused the appearance of external injury symptoms but also affected internal quality and chemical composition of the commodity. In situations where outer parts of the product are removed during marketing such as in most fresh-cut items, internal quality of the chilling-sensitive commodities can be negatively affected by chilling temperatures even though typical chilling injury symptoms such as surface pitting are not present. Treatments that can alleviate chilling injury should also improve the internal quality of these chilling-sensitive fresh produce as well as that of fresh-cut products.

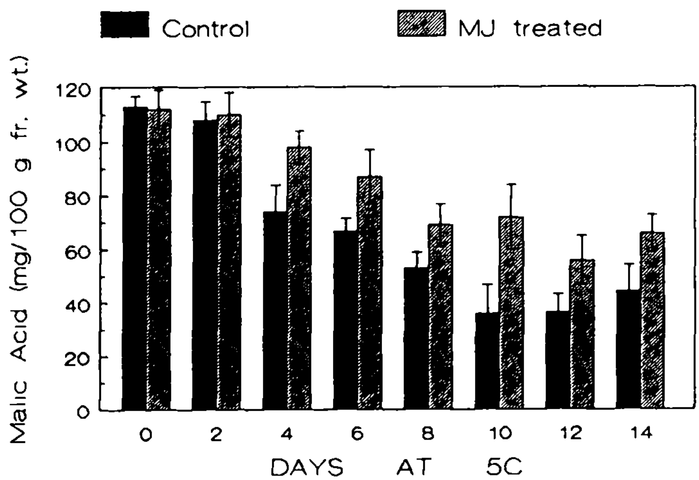

FIG. 4. EFFECT OF 0.1 mM METHYL JASMONATE (MJ) ON CHANGES OF MALIC ACID CONTENT IN ZUCCHINI SQUASH DURING STORAGE AT 5C
Vertical bars represent SE with n = 3.

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