

Effect of Low-Oxygen Storage on Chilling Injury and Polyamines in Zucchini Squash

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ABSTRACT

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Chilling injury of Zucchini squash (*Cucurbita pepo* L., cultivar 'Ambassador') was reduced by a low-oxygen atmosphere. The extent of surface pitting after exposure to 2.5 °C was less under 1% O₂ than when kept in air. The low-oxygen-stored squash also maintained higher levels of spermidine and spermine during storage at 2.5 or 12.5 °C. Putrescine and spermidine in the skin tissue increased in response to exposure to chilling temperature. However, no significant increase of these polyamines was detected during storage at the nonchilling temperature.

Keywords: *Cucurbita pepo*; pitting; putrescine; spermidine; spermine.

INTRODUCTION

The effectiveness of controlled atmospheres in reducing chilling injury varies with the commodity, the maturity, the concentrations of O₂ and CO₂, the duration of treatment, and the storage temperature (Wang, 1982). Beneficial effects of using low-oxygen and high-carbon dioxide atmospheres to reduce the development of chilling injury in Zucchini squash have been reported (Mencarelli et al., 1983; Mencarelli, 1987). Marketability of the squash was higher after storage in 1-4% O₂ + 0% CO₂ at 2.5 °C or in 21% O₂ + 5% CO₂ at 5 °C than after storage in air. It appears that the modification of storage atmospheres maintains better storage quality of Zucchini squash at chilling temperatures. Although low-oxygen and high-carbon dioxide atmospheres have been shown to affect respiration, ethylene production and action, organic acids, carbohydrates and other metabolites (Kader, 1985; Wang, 1988a), the mechanism through which controlled atmospheres alter chilling sensitivity is not clear.

Recently, 1% O₂ was found to maintain higher levels of polyamines in Chinese cabbage during storage at 0 °C (Wang, 1988b). In both outer and inner lami-

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nae, higher endogenous concentrations of polyamines seemed to be associated with the delay of senescence in low-oxygen-stored Chinese cabbage. Polyamines have been shown to affect DNA and RNA synthesis and degradation, to regulate the rate of transcription, to stabilize ribosomal structures and to maintain membrane integrity (Mager, 1959; Cohen, 1971; Bachrach, 1973). However, it is not known whether polyamines play a role in reducing chilling injury. The present study was initiated to determine the effect of 1% O₂ on polyamine levels in Zucchini squash and to evaluate the relationship between polyamine levels and the degree of chilling injury.

MATERIALS AND METHODS

Zucchini squash were freshly harvested from a local farm near Beltsville, MD. Samples were selected for their uniformity of size (20–22 cm in length). They were randomly divided into two lots and immediately stored at 2.5 (chilled) or 12.5 °C (nonchilled). Within each temperature, the squash were further divided into two lots and stored in two different atmospheres (air or 1% O₂).

Stainless steel chambers (200 l) with glass doors were used as storage containers. Forty-five squash were stored in each chamber for each treatment. To establish 1% O₂ atmosphere, the sealed chambers were flushed initially with nitrogen at high rates, then followed with 1% O₂ from premixed gas cylinders. Flow rate was maintained at 100 ml min⁻¹ throughout the storage. For air storage, the sealed chambers were flushed with air from compressed air cylinders at the same flow rate. The 1% O₂ atmosphere was verified and monitored by an Orsat and a gas chromatograph (Shimadzu) equipped with a thermal conductivity detector. Approximately 300 g of Purafil and 500 g of lime were placed in each chamber to absorb ethylene and carbon dioxide. A pan of water was also placed on the bottom of each chamber to maintain the relative humidity at above 95% (monitored with a Cole-Parmer thermocouple psychrometer).

Samples were taken initially and after 3, 6, 9, 12 and 15 days of storage for analysis of polyamines. The 1% O₂ atmosphere was re-established as described above immediately after each sampling. The extraction was performed 5 h after the transfer of squash from storage chambers to 20 °C in air. Polyamines were analyzed according to a previously described procedure with some modifications for squash skin tissue (Wang, 1988b). Two g of skin tissue were extracted with 15 ml of 5% ice-cold perchloric acid. To each sample, 20 µl of 50 mM 1,6-hexane diamine was added as internal standard. The homogenate was centrifuged at 5000 *g* for 20 min. Dansyl chloride was added to the supernatant and allowed to stand in the dark overnight. The dansylated products were then extracted with benzene and separated on silica gel thin layer plates (Whatman LK6D) at 5 °C. The solvent system used was cyclohexane:ethylacetate (5:4, v/

v). The fluorescent spots corresponding to putrescine, spermidine and spermine were eluted with ethyl acetate and were quantified with a HTV Fluoroflow Detector V spectrophotofluorometer with excitation at 350 nm and emission at 495 nm.

The degree of chilling injury for each squash was evaluated before the extraction for polyamines. The severity of the injury was rated on a scale of 1–5 with 1 = no abnormality and 5 = severe chilling injury. The experiment was replicated three times with 45 fruits treatment⁻¹ each time.

RESULTS

After 3 days of exposure to 2.5 °C, Zucchini squash from both air and 1% O₂ storage appeared normal without symptoms of chilling injury (Fig. 1). However, after 6 days at 2.5 °C, slight pitting was found on the skin of squash from air storage, whereas those from 1% O₂ storage remained normal. The development of chilling injury symptoms progressed rapidly in squash kept in air. Severe pitting and slight decay were observed in these squash after 12 days of exposure to chilling temperature. The development of surface pitting was slower in squash kept in 1% O₂ atmosphere. The difference in the severity of chilling injury between air-stored and 1% O₂-stored squash was evident after 6 days of exposure to 2.5 °C. Squash kept under 1% O₂ atmosphere developed moderate to severe pitting only after 15 days of exposure to chilling temperature. Decay was absent in squash held in 1% O₂ throughout the experiment.

Putrescine increased in response to chilling exposure in samples stored both in air and in 1% O₂ (Fig. 2). Spermidine also increased in the skin tissue after the exposure to chilling temperature (Fig. 3). The initial increase of spermidine was followed by an overall decline in air-stored samples after 6 days of

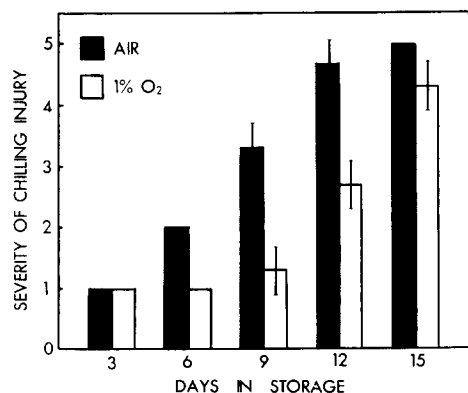


Fig. 1. Effect of 1% O₂ on chilling injury (1 = normal, 5 = severe) at 2.5 °C in Zucchini squash. Vertical bars indicate \pm SE.

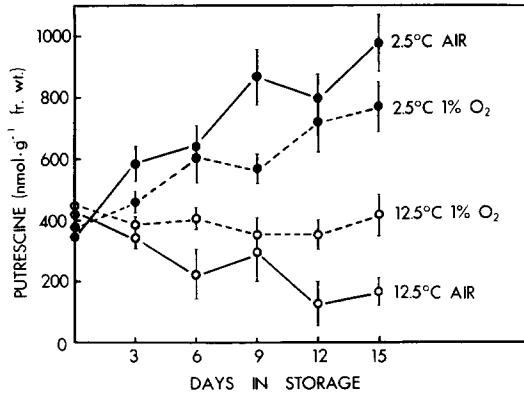


Fig. 2. Effect of 1% O₂ on putrescine content in Zucchini squash stored at 2.5 or 12.5°C. Vertical bars indicate \pm SE.

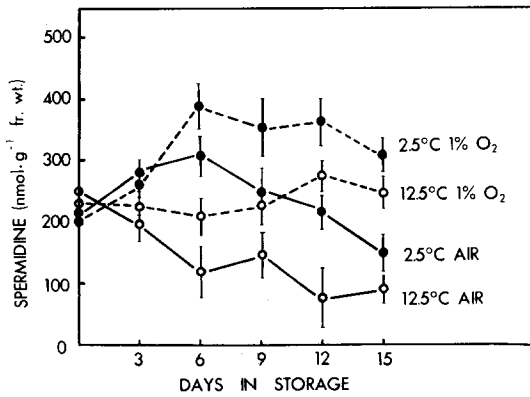


Fig. 3. Effect of 1% O₂ on spermidine content in Zucchini squash stored at 2.5 or 12.5°C. Vertical bars indicate \pm SE.

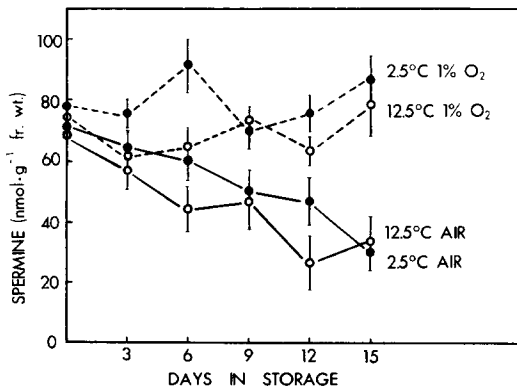


Fig. 4. Effect of 1% O₂ on spermine content in Zucchini squash stored at 2.5 or 12.5°C. Vertical bars indicate \pm SE.

exposure to 2.5°C. The squash stored under 1% O₂ maintained higher levels of spermidine. At 12.5°C, putrescine and spermidine in air-stored samples decreased with time in storage. However, the decrease was suppressed by 1% O₂. Concentrations of spermine in the skin of Zucchini squash (Fig. 4) were relatively low compared to those of putrescine and spermidine (Figs 2 and 3). Spermine decreased during storage in air but remained at consistently higher levels under 1% O₂ at both 2.5 and 12.5°C. The spermine levels did not appear to be stimulated by chilling temperature.

DISCUSSION

Accumulation of putrescine in the tissue occurred upon exposure of Zucchini squash to chilling temperature (Fig. 2). The results are consistent with those observed in chilled bean plants (Guye et al., 1986) and grapefruit, pepper and lemon fruits (McDonald and Kushad, 1986). The increase in putrescine levels seems to be a general reaction of plants to various kinds of stress, including water stress (Wang and Steffens, 1985), acid stress (Smith and Sinclair, 1967; Young and Galston, 1983), osmotic shock (Flores and Galston, 1982) and mineral deficiency (Coleman and Richards, 1956; Smith, 1985). Although some studies have correlated this increase to an enhanced activity of arginine decarboxylase (Galston, 1983), there is no general consensus as to the role of the elevated level of putrescine during stress. Guye et al. (1986) showed that polyamine levels increased upon chilling in hardened bean plants and postulated that the changes in putrescine content may be correlated with chill tolerance. On the other hand, McDonald and Kushad (1986) reported a significant correlation between putrescine concentrations and the severity of chilling injury in lemons, grapefruit and peppers, and proposed that the accumulation of putrescine in the chilled tissues could be a cause of stress-induced injury. Phelps and McDonald (1989) found that abnormally high concentrations of spermidine and spermine might be detrimental to several oxidase activities, especially to external nicotinamide adenine dinucleotide oxidase in pepper and avocado fruit mitochondria.

In 1% O₂, Zucchini squash maintained higher levels of spermidine and spermine than in air storage (Figs 3 and 4). The triamine spermidine and the tetramine spermine are thought to be more effective in antisenesescence activity than the diamine putrescine because they possess a greater number of amine groups in the molecule (Galston and Kaur-Sawhney, 1987). Since Zucchini squash stored in 1% O₂ atmosphere had less chilling injury than those stored in air, these polyamines may be involved in reducing chilling injury in Zucchini squash. Nevertheless, direct evidence for the cause and effect on polyamines and chilling injury is lacking. Further studies are needed to clarify the relationship between polyamines and chilling injury.

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