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Modified atmosphere packaging alleviates chilling injury in cucumbers

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Abstract

Cucumbers *(Cucumis sativus* L.) packaged in perforated or sealed 31.75 μm (1.25 mil) low density polyethylene (LDPE) bags were found to have less severe chilling injury than nonwrapped fruit in storage at 5°C and 90–95% relative humidity. The onset of chilling injury was also delayed by the LDPE packaging compared to the nonpackaged control. The concentrations of CO_2 increased to 3% while O_2 levels decreased to 16% in the sealed bags. Fruit in the sealed bags had the least decay. The O_2 and CO_2 concentrations inside the perforated bags changed very little from the ambient atmosphere. However, there was a marked difference in the weight loss between nonwrapped cucumbers and fruit from perforated or sealed bags. The weight loss of nonwrapped fruit reached 9% in 18 days while perforated and sealed samples lost less than 1% during the same period. Chilling stress induced increases in putrescine levels in all treatments but the sealed fruit had the highest levels of putrescine. Sealed fruit and perforated fruit also had higher content of spermidine than non-wrapped fruit. These high levels of polyamines may have contributed to the increase of chilling tolerance in fruit from perforated and sealed packages. © 1997 Elsevier Science B.V.

Keywords: Chilling injury; Cucumbers; *Cucumis sativus*; Packaging

1. Introduction

Packaging offers protection to products from physical, physiological, and pathological deterioration throughout marketing (Hardenburg, 1966). Major benefits of film packaging of fruits and vegetables include reduction of moisture loss and modification of in-package atmosphere (Hardenburg, 1971). Thus, film packaging has been used as a supplement to refrigeration to extend storage and shelf life of many fruits and vegetables (Hardenburg et al., 1986). For crops which are sensitive to chilling temperatures, the increase in humidity and the reduction in O_2 concentration and accumulation of CO_2 within the package often are beneficial for preventing the development of chilling injury symptoms (Forney and Lipton, 1990).

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One of the major effects of packaging is the maintenance of high humidity surrounding the products. The advantage of high humidity in suppressing chilling injury has been shown by several researchers (Wang, 1993). Brooks and McColloch (1936) reported that grapefruit stored at 4.5°C for 8–10 weeks exhibited significantly higher percentage of pitting under lower relative humidity (65–75%) than higher humidity (85–90%). Morris and Platenius (1938) showed that cucumbers stored at 5°C for 7 days developed severe pitting in 50–60% relative humidity, while the pitting was prevented in 95–100% relative humidity. Severe chilling injury was also evident in sweet peppers after 13 days of storage at 4.5°C in 65–75% relative humidity, while no chilling injury was observed in 98–100% relative humidity at the same temperature (Morris and Platenius, 1938). Increasing the humidity in the storage environment has also resulted in reducing chilling injury of limes and grapefruit (Pantastico et al., 1968; Wardowski et al., 1973).

Another major effect of film packaging is the modification of atmospheric composition within the package. Changes in O_2 and CO_2 concentrations in the atmosphere surrounding the commodities can influence the chilling susceptibility (Forney and Lipton, 1990). Increase in CO_2 and decrease in O_2 levels have been shown to reduce chilling injury of avocados, grapefruit, peaches, nectarines, okra, pineapples, potatoes, and zucchini squash (Wang, 1993). However, elevated CO_2 concentrations were found to increase the symptoms of chilling injury in cucumbers at 5°C (Eaks, 1956). It is not known if the CO_2 accumulated in the film package would adversely affect the sensitivity of cucumbers to chilling. This study was initiated to evaluate the effect of modified atmosphere packaging on chilling susceptibility of cucumbers.

2. Materials and methods

2.1. Plant material and packaging treatments

Cucumbers *(Cucumis sativus* L., cv. Thunder) were freshly harvested from a farm in southern Maryland. Fruit uniform in size and free from blemishes were chosen for the experiment. They were randomly divided into three lots and used for three packaging treatments: nonwrapped, perforated, and sealed.

Low density polyethylene (LDPE) bags (33.5 × 35 cm in size) were used for packaging. The thickness of polyethylene film was 31.75 μm (1.25 mil). Ten holes (each 6 mm in diameter) were made in the perforated bags. The amount of perforation represented approximately 0.25% of the total surface area of the bag.

2.2. Storage conditions and chilling injury evaluation

All cucumbers were placed into 5°C storage with 90–95% relative humidity immediately after harvest. Packaging treatments were carried out after 24 h at 5°C to avoid excessive accumulation of CO_2 in the sealed bags due to high respiration rate of warm fruit from the field. Three fruits were placed in each bag and three bags were removed at each sampling for evaluation of chilling injury and chemical analyses.

The severity of chilling injury was evaluated 24 h after cucumbers were transferred from 5°C to 20°C. The degree of chilling injury, as judged by the extent of surface pitting, was rated on a scale of 1–5, with 1, no abnormality; 2, trace; 3, slight; 4, moderate; and 5, severe chilling injury.

2.3. Measurement of weight loss and analysis of in-package atmosphere

Individual cucumbers were weighed before the packaging treatments and again at removal from 5°C to determine the effect of different packaging treatments on weight loss during storage at 5°C. After evaluation of chilling injury and weighing, two 2 g samples of exocarp tissue were removed from each fruit and immediately placed in a −80°C freezer. A 3 ml gas sample was taken with a syringe from each package atmosphere before opening to analyze the in-package O_2 and CO_2 concentrations. Gas analyses were performed with a Shimadzu gas chromatograph.

2.4. Analysis of polyamines

Exocarp samples (2.0 g) were homogenized in 15 ml 5% perchloric acid with a Polytron homogenizer. 1,6-Hexanediamine (500 nmol/g fresh wt.; Sigma) was added as an internal standard. The homogenate was then centrifuged at 47 000 g for 20 min. The supernatant was used for polyamine determination. Polyamines were analyzed using HPLC methods similar to those of Smith and Davies (1985). Dansylation was performed by mixing 400 μl dansyl chloride (18.5 mM in acetone; Sigma) and 150 μl saturated sodium carbonate with 200 μl of extract. After incubation overnight at room temperature, 200 μl proline (0.43 M) were added to remove excess dansyl chloride and incubation was continued for 1 h. After centrifugation for 10 min in a microcentrifuge (Beckman), the pH of the supernatant was checked and neutralized to pH 7.0 with HCl. A 100 μl sample of supernatant was used for HPLC analysis.

HPLC was performed on a system (Waters) consisting of 2 Model-510 pumps (Waters) programmed with a 720 System Controller (Waters). Samples were injected using a Rheodyne injector into a reverse phase C-18 column (Supelco 25 cm LC-18 with a Supelguard LC-18 5-μm guard column). Samples were eluted from the column at a flow rate of 1.5 ml/min with a programmed solvent gradient of 0, 100, 0; 15, 0, 100; 19, 0, 100; 19.1, 100, 0; where the first number is the time (min), the second number the percentage of buffer A (60:40, v/v, methanol: water), and the third number the percentage of buffer B (100% methanol). This program was found to be the best solvent gradient for the separation of polyamines in cucumbers. Elution was completed in 19 min. Eluates were detected by a 1046 A programmable fluorescence detector (Hewlett Packard) using an excitation wavelength of 365 nm and an emission wavelength of 510 nm. Data were collected and analyzed using a Compaq 286 computer system equipped with a Baseline 810 Chromatography Workstation (Dynamic Solutions). Each data point is the average of three independent samples.

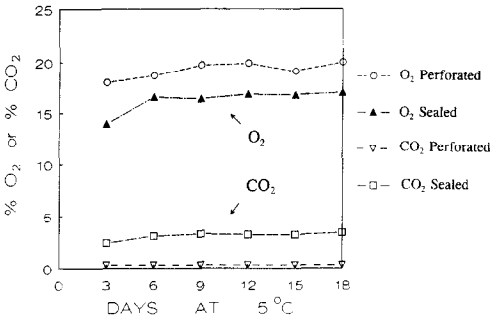

Fig. 1. Concentrations of O_2 and CO_2 in perforated or sealed packages of cucumbers during storage at 5°C.

3. Results and discussion

Measurement of the in-package atmosphere revealed that CO_2 concentrations in the perforated bags were maintained around 0.12– 0.16% and did not rise to more than 0.2% (Fig. 1) The CO_2 concentrations in the sealed bags, however, accumulated gradually during the first 7 days to 3.0% and then leveled off thereafter. The O_2 levels in the perforated bags remained at around 19–20% throughout the experiment (Fig. 1). Slightly lower levels of O_2 (16–17%) were detected in the sealed bags.

A marked difference in weight loss was found between nonwrapped samples and other treatments after 5 days of storage at 5°C (Fig. 2). The difference became even more pronounced as storage progressed. After 18 days of storage, the nonwrapped samples lost 9.2% while fruit from perforated bags lost 0.9% and those from sealed bags lost only 0.2%. Apparently, polyethylene film

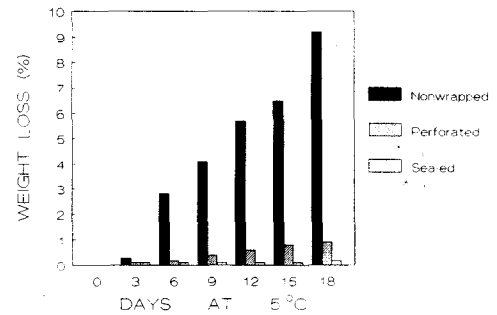

Fig. 2. Effect of modified atmosphere packaging on weight loss of cucumbers during storage at 5°C.

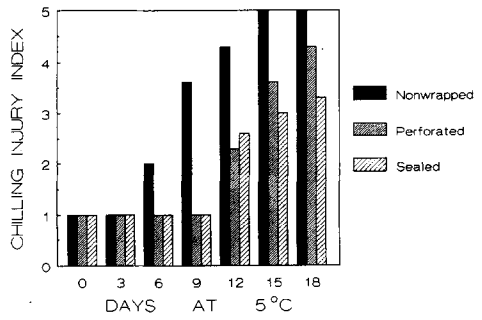

Fig. 3. Effect of modified atmosphere packaging on the development of chilling injury (1, normal; 5, seveve) in cucumbers during storage at 5°C.

with perforations was still a good barrier to moisture transfer even though it did not restrict gas exchange. Shrivel in fresh fruits and vegetables becomes obvious when weight loss reaches 3–6% (Hardenburg et al., 1986; Hruschka, 1977; Lentz, 1966). The symptoms of shrivelling in nonwrapped cucumbers in this experiment were not only apparent but also objectionable after 12 days of storage (data not shown).

Chilling injury symptoms started to develop in nonwrapped cucumbers by the 6th day of storage at 5°C (Fig. 3). The symptoms first appeared as numerous tiny pits then advanced to large sunken spots and scattered water-soaked areas. These symptoms progressed rapidly in non-wrapped samples with increasing duration of storage. Symptoms became more pronounced after fruit were transferred from 5° to 20°C. Lesions of decay also developed over the surface of nonwrapped fruit by the 12th day of storage. The onset of chilling injury symptoms in cucumbers in perforated or sealed packages were delayed (Fig. 3). However, decay also developed in fruit in perforated bags by the 15th day. No rotten spots were observed on fruit from sealed packages. Fruit from sealed packages did not exhibit any sign of CO_2 injury or off-flavor when tasted at the end of the experiment.

Eaks (1956) reported that concentrations of CO_2 3% or higher seemed to increase pitting and accelerate the rate of breakdown in cucumber fruit held at 5°C. In the present study, CO_2 concentrations inside the sealed packages remained at around 3% after 7th day of storage at 5°C. The combination of elevated CO_2 and the near saturated humidity within the package apparently delayed the development of pitting and suppressed the growth of decay pathogens in our experiment.

High polyamine levels have been correlated with increased chilling resistance in zucchini squash (Kramer and Wang, 1989). It has been hypothesized that polyamines may protect the integrity of membranes which in turn alleviates chilling injury. In order to determine if polyamines were involved in changes in chilling sensitivity of cucumbers induced by modified atmosphere packaging, the endogenous levels of putrescine, spermidine, and spermine were analyzed. Putrescine increased gradually during storage at 5°C in all three treatments from the initial level at harvest (Fig. 4). Accumulation of putrescine in tissues seems to be a general response of plants to chilling temperatures (Guye et al., 1986; Kramer and Wang, 1989) and other stresses (Flores, 1990). Chilling-induced stimulation of putrescine synthesis is correlated with increased ornithine decarboxylase activity (Kramer and Wang, 1990). This indicated that the ornithine decarboxylase pathway was used to synthesize chilling-induced putrescine instead of the arginine decarboxylase pathway as in the case of most other stresses (Smith, 1985). Putrescine levels in cucumbers from the sealed bags was significantly

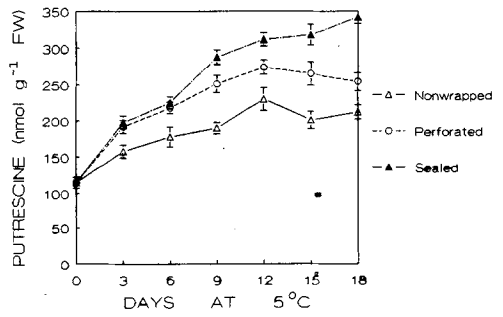

Fig. 4. Effect of modified atmosphere packaging on putrescine content in cucumbers stored at 5°C. Error bars represent ± S.E.

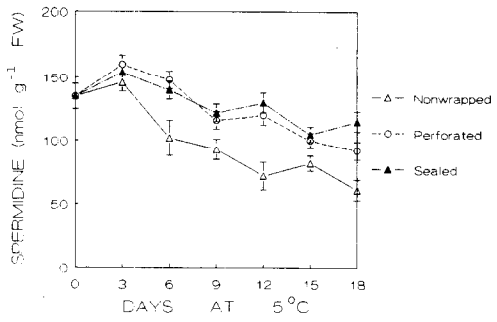

Fig. 5. Effect of modified atmosphere packaging on spermidine content in cucumbers stored at 5°C. Error bars represent ± S.E.

higher than those from perforated bags or the nonwrapped samples particularly during later part of storage (Fig. 4). Additional stress imposed by elevated CO_2 concentration within sealed packages might have contributed to this high level of putrescine. Similar accumulation of putrescine in cucumbers in response to CO_2 stress has previously been reported (Mathooko et al., 1995). Spermidine levels decreased steadily in fruit from nonwrapped treatment during storage (Fig. 5). Spermidine in samples from perforated or sealed bags also declined during storage but remained at higher levels than those in nonwrapped fruit. Higher levels of spermidine and spermine are correlated with reduced chilling injury in temperature preconditioned zucchini squash (Kramer and Wang, 1989). Spermine contents were relatively low in cucumbers compared to putrescine and spermidine. The levels of spermine fluctuated widely during storage and no consistent differences were found among the various treatments. Nevertheless, the higher levels of putrescine and spermidine may have contributed to the reduced chilling injury in cucumber fruit from perforated and sealed packages.

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