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CONTROLLED ATMOSPHERE STORAGE SUPPRESSES MICROBIAL GROWTH ON FRESH-CUT WATERMELON

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Abstract. **The increasing consumption of fresh-cut produce has led researchers and industry to look for techniques that can increase postharvest life of these products while assuring safety and quality. Controlled atmosphere storage has been used to increase storage life of fresh fruits and vegetables since it slows physiological processes and suppresses microbial growth. Seedless watermelons (*Citrullus lanatus* Thumberg) were cut in 2.5 cm cubes and stored at 3°C for 15 days under five different atmospheres: air, 3% O_2, 3% O_2 + 5% CO_2, 3% O_2 + 10% CO_2, 3% O_2 + 15% CO_2, 3% O_2 + 20% CO_2(balance nitrogen). The concentrations of 3% O_2 + 15% CO_2 and 3% O_2 + 20% CO_2 inhibited bacterial development during the entire storage time, but had negative effects on the visual quality of the cubes.**

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Introduction

The fresh-cut produce industry has shown a pattern of increasing market size in the United States. In the last ten years, several studies have revised projections for the growth of this industry. In 1992, Hurst and Schuler mentioned that the fresh-cut industry predicted a market of $4 to 8 billion by the year 2000. More recently, however, Hodge (1995) noted that, by 1999, this industry will have annual sales of about $19 billion.

Watermelon (*Citrullus lanatus*) production in Florida is also on the rise. According to the Florida Agricultural Statistics Service (1997), the state produced 714 million lb for the 1995-1996 season, corresponding to $50 million, with an increase in planted acreage over previous seasons.

With the increase in consumption of fresh produce, quality is a concern of researchers and industry in this field. Fresh-cut products are more perishable than the intact counter part, a consequence of the physical stresses associated with the processing techniques. Even the removal of the epidermis of a fruit or vegetable represents physical damage to the tissue, which becomes subject to intense physiological changes. These changes, in addition to microbial contamination, significantly shorten postharvest life of produce (King Jr. and Bolin, 1989; Cantwell, 1992; Cantwell, 1995a; Brecht, 1995; Schlimme, 1995; Watada et al., 1996).

Maximizing shelf life of fresh-cut produce has been a major focus in the food industry, and microbial status is one of the key limiting factors, making sanitation of critical concern (Bracket, 1992; Hurst, 1995). Food safety is even more important for fresh-cut produce since it does not undergo thermal treatments (Reyes, 1996). Microbial growth is usually favored in fresh-cut produce by increased surface area, high moisture content inside packages, and by handling and preparation processes (Brackett, 1987; Brackett, 1993; Nguyen-the and Carlin, 1994; Cantwell, 1995b).

Spoilage and decay of fresh-cut produce are often reported by researchers working in this area. Robbs et al. (1996) attributed the light green and brown discoloration of fresh-cut celery stored in sealed bags for 21 days at 2°C to bacterial decay, while Izumi et al. (1996) noted off-flavor development in sliced zucchini after 10 days of storage in air at 10°C. Watermelon is reported to be extremely perishable upon slicing, which often limits postharvest life to 1 to 2 days at retail level, necessitating preparation in the produce department.

Controlled atmosphere (CA) storage has been recently used on fresh-cut fruits and vegetables as a means to increase postharvest life. Metabolic activities are reduced which leads to a suppression of microbial growth, an alleviation of chilling injury and a slowing of senescence. However, if misused, CA can also promote physiological injury and cause off-flavor due to anaerobic respiration or excessive CO_2 concentrations (Kader, 1992; Cantwell, 1995b).

CA has been shown to be very effective in controlling decay in fresh-cut cantaloupe held for 10 days at 7.5°C (Madrid and Cantwell, undated), increasing shelf-life of diced cantaloupe up to 28 days (O'Connor-Shawn et al., 1996) and increasing shelf-life of celery stalks to 10 weeks by reducing black stem (Smith and Reyes, 1988). However, despite the decay control achieved in CA-stored cantaloupe (Madrid and Cantwell, undated), off-odors and off-flavors occurred after 5 days at 7.5°C. Brief immersions of whole cantaloupe into boiling water significantly reduced pathogens on the fruit surfaces prior to slicing (O'Connor-Shawn et al., 1996).

Considering the benefits and limitations of CA storage, the objective of this study was to determine the ideal O_2 and CO_2 concentrations for fresh-cut watermelon, in order to increase the postharvest life of this product by minimizing microbial growth while maintaining visual and sensory quality.

Materials and Methods

Seedless watermelons (cv. Millionaire) were obtained 4 days after harvest, washed with tap water and stored for 36 hours at 3°C. Twelve hours prior to cutting, the fruit were rinsed with chlorinated water containing 150 ppm free chlorine as were the utensils and cutting surfaces, and coldroom interior. Hairnets, latex gloves, surgical masks, and disposable aprons were worn during cutting and handling in order to minimize contamination.

The fruit were sorted by soluble solids content (≥7°Brix) obtained from the blossom end prior to slicing at 3°C. The rind was removed from each fruit and the flesh was cut in 2.5 cm cubes with sharp knives. The cubes were randomized, stored in sealed glass jars (n = 10 cubes/jar) with humidified flow-through air or controlled atmospheres and held at 3°C. The CA mixtures were: air; 3% O_2; 3% O_2 + 5% CO_2; 3% O_2 + 10% CO_2; 3% O_2 + 15% CO_2; 3% O_2 + 20% CO_2, with the balance consisting of N_2.

Samples were taken after 5, 10 and 14 days for each treatment to measure color, firmness, total soluble solids (TSS) content, titratable acidity (TA), pH, juice leakage (n = 3 jars) and after 15 days for microbial analysis (n = 2 jars).

At each sampling time, juice leakage was calculated for each of the jars by the ratio of juice weight/tissue weight and expressed in µg of juice/g of tissue. Five cubes were removed from each of the three replicate jars for color measurement. L*, a*, and b* values were read using surface reflectance by a chroma meter (Model CR-200, Minolta, Japan), and a* and b* values were later transformed to hue angle (color) and chroma (color intensity) values. Cubes with uniform size were used for firmness measurement by puncture analysis with a 10 mm convex tip (Instron Universal Testing Instrument, Model 1132, Canton, MA), a crosshead speed of 10 cm min^{-1} and full scale of 5 kgf. Values were converted to N. Samples for TSS, pH, and TA, were first blended and centrifuged at 15,000 rpm for 15 minutes. The TSS of the supernatant was determined by refractometry (Reichert-Jung, Mark Abbe II Refractometer, Model 10480, Depew, NY). An automatic titrimeter (Fisher Titrimeter II, No. 9-313-10, Pittsburgh, PA) was used to determine % acid.

Two methods were employed for determination of bacterial number: pour plate for samples containing low bacterial numbers, and spread plate for samples with high bacterial loads. Four to five cubes of watermelon (70 g) were randomly selected from each treatment, placed in each of two sterile jars and homogenized. For pour plate, 1.0 or 0.1 ml of the fruit homogenate was transferred to an empty petri dish then mixed with cooled PCA media. For spread plate, the homogenate was spread on plate count agar (Difco Laboratories, Detroit, MI) immediately after homogenization. For both methods, four plates were used for each dilution. Bacteria colonies were counted after 2 to 3 days of incubation at room temperature (±25°C).

The data were analyzed using SAS GLM and Duncan's multiple range test.

Table 1. L* value, Hue angle and Chroma for watermelon cubes stored under several atmospheres at 3°C at four sampling dates.

Treatment	L* Value				Hue Angle				Chroma			
	Day 1[z]	Day 5	Day 10	Day 14	Day 1	Day 5	Day 10	Day 14	Day 1	Day 5	Day 10	Day 14
Air	40.90 b	47.09 a	44.97 a	43.11 a	37.54 a	40.57 ab	41.36 a	43.32 a	30.74 a	29.16 a	30.48 a	28.19 ab
3% O_2	44.55 ab	43.04 ab	42.41 a	44.48 a	40.33 a	41.70 a	39.09 a	40.46 ab	27.03 a	27.75 a	29.23 a	29.16 ab
3% O_2/5% CO_2	44.37 ab	42.07 b	44.35 a	45.55 a	38.73 a	37.86 b	38.49 a	41.67 ab	28.70 a	30.73 a	30.04 a	27.38 b
3% O_2/10% CO_2	45.51 a	44.95 ab	41.65 a	43.38 a	39.65 a	38.08 b	38.96 a	39.03 b	30.72 a	31.01 a	30.03 a	31.20 a
3% O_2/15% CO_2	45.08 a	44.33 ab	41.81 a	42.48 a	38.80 a	39.32 ab	38.43 a	40.36 ab	30.99 a	27.57 a	28.05 a	28.05 ab
3% O_2/20% CO^2	43.59 ab	44.67 ab	43.45 a	43.89 a	38.13 a	39.52 ab	41.30 a	39.17 b	30.09 a	29.98 a	28.32 a	28.32 ab

[z]Values in the same column not followed by the same letter are significantly different at $P \leq 0.05$.

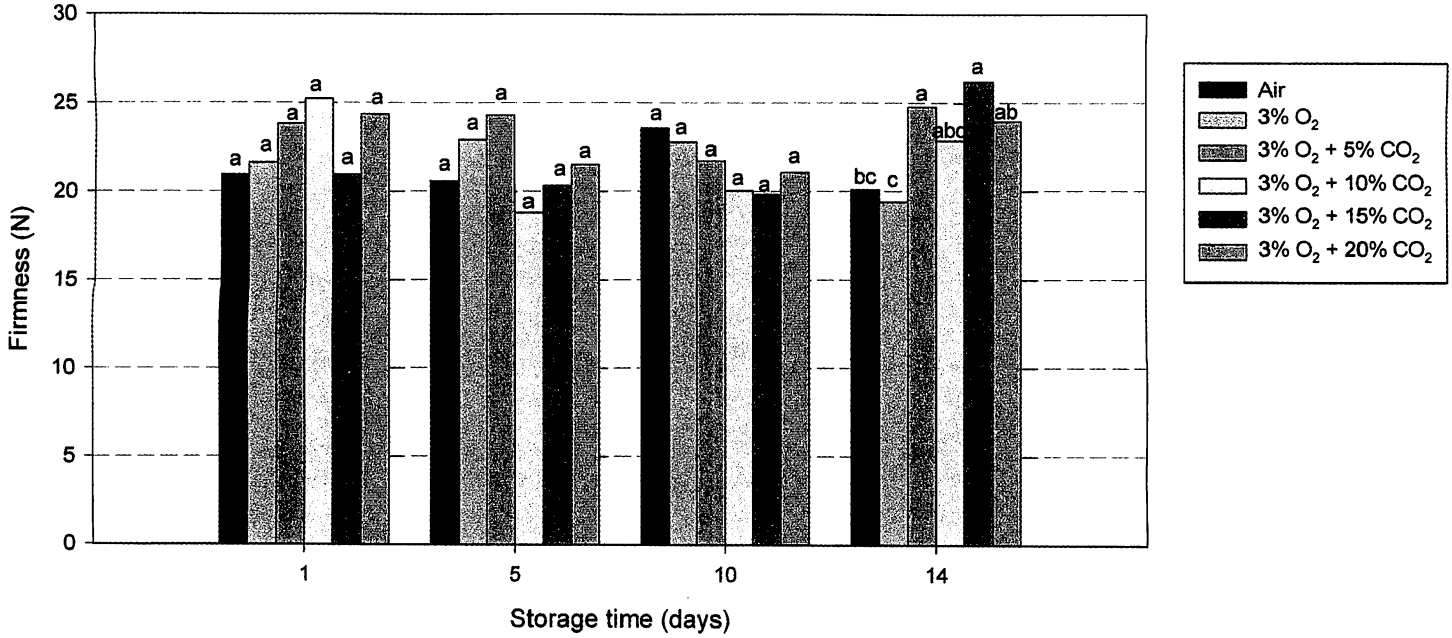

Figure 1. Changes in firmness of fresh-cut watermelon stored under several atmospheres for 14 days at 3°C. Mean separation by Duncan's Multiple Range Test; means with the same letter for each storage day are not significantly different ($P \leq 0.05$).

Results and Discussion

The color of watermelon cubes changed significantly over time ($P \leq 0.05$). Hue angle for samples stored in air increased significantly, becoming orange-red following 5 days of storage. Although there was no statistical change in hue angle for the high CO_2 treatments, cubes stored in 3% O_2 + 10% CO_2, 3% O_2 + 15% CO_2, and 3% O_2 + 20% CO_2 appeared darker and somewhat watersoaked at the end of the experiment, possibly indicating a symptom of low O_2 or high CO_2 toxicity on the watermelon tissue. Extreme atmospheres may also have enhanced juice leakage in the cubes under those treatments. Chroma decreased significantly (became duller) in tissue stored at 3% O_2 + 5% CO_2 between days 5 and 14, but showed no change in the other treatments. L* values were similar for all treatments after 14 days storage, with values ranging from 45.5 for tissues in 3% O_2 + 5% CO_2, to 42.48 for samples in 3% O_2 + 15% CO_2 (Table 1).

Tissues stored under air and 3% O_2 for 14 days tended to become significantly softer than samples stored in 3% O_2 + 5% CO_2 and 3% O_2 + 15% CO_2 (Fig. 1). Tissues in air had significantly higher Brix than samples in 3% O_2 + 15% CO_2 and

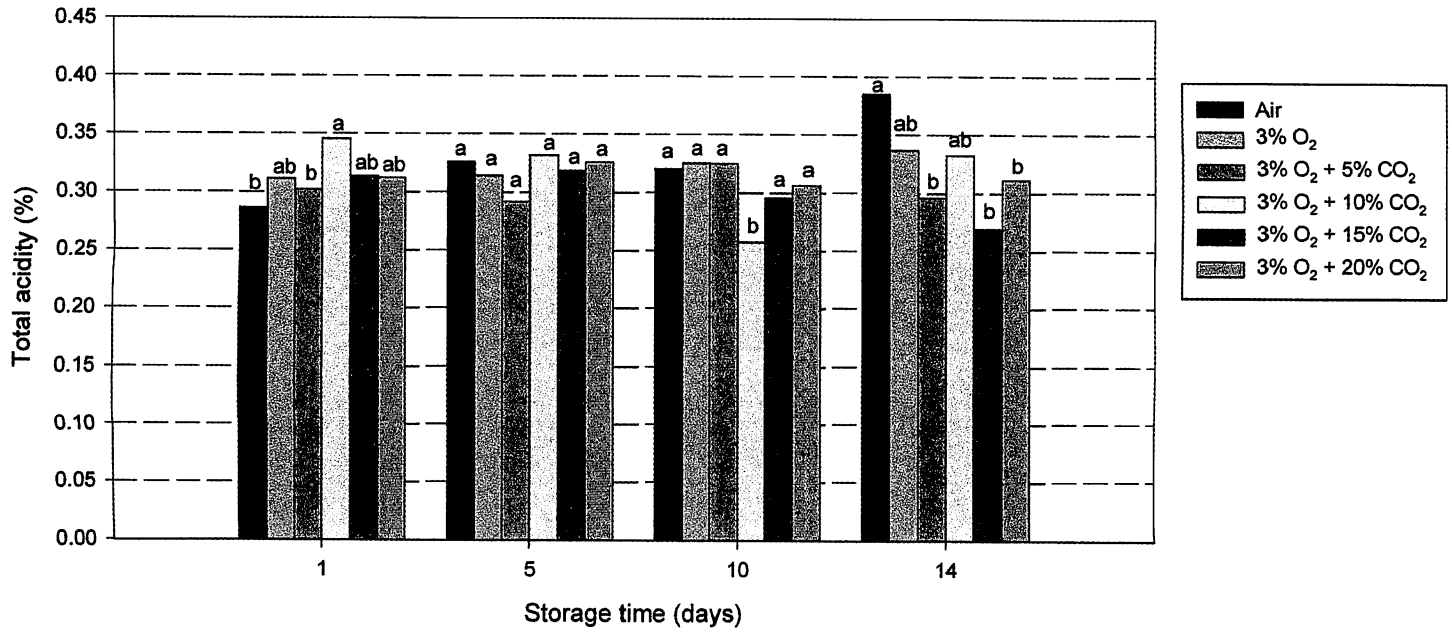

Figure 2. Titratable acidity changes in fresh-cut watermelon stored under several atmospheres for 14 days at 3°C. Mean separation by Duncan's Multiple Range Test; means with the same letter for each storage day are not significantly different ($P \leq 0.05$).

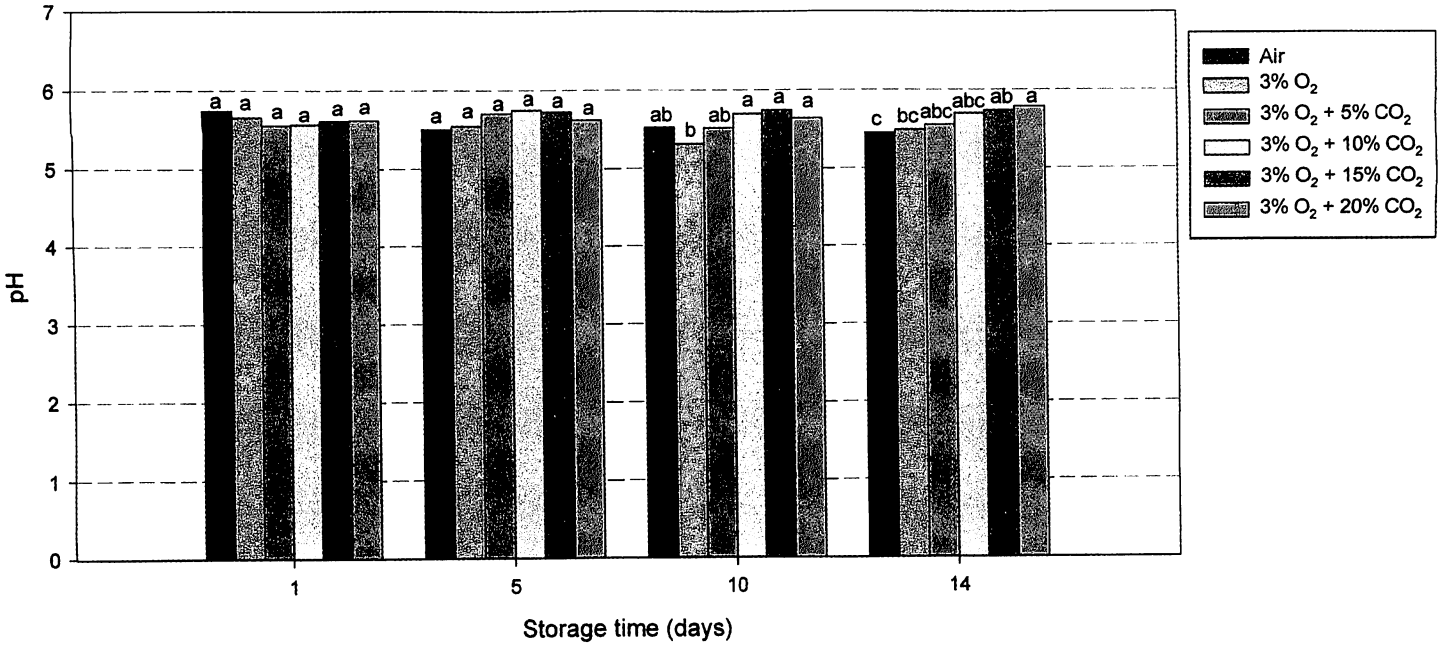

Figure 3. Changes in pH of fresh-cut watermelon during storage at 3°C under several atmospheres for 14 days at 3°C. Mean separation by Duncan's Multiple Range Test; means with the same letter for each storage day are not significantly different ($P \leq 0.05$).

3% O_2 + 20% CO_2 after 10 days of storage being values of 10.33, 7.90 and 8.03, respectively, after 14 days (data not shown). Titratable acidity increased for the air treatment after 5 days of storage (Fig. 2) accompanied by a corresponding decrease in pH (Fig. 3). This trend can be explained by an increase in organic acid content during the storage time and/or microbial proliferation.

Despite the significant increase in juice leakage over storage time for samples stored in air and 3% O_2 + 15% CO_2, tissues stored in air had less juice accumulation than all the other treatments, with 3% O_2 + 20% CO_2 showing the largest volume of juice after 10 days of storage (Fig. 4).

The undesirable effects of non-air storage treatments on watermelon quality, may have been physiological injury responses to low O_2 at 3%. Low-oxygen injury has been documented for a number of crops, being characterized by the development of off-odors and off-aromas (Zagory, 1995; Huxoll and Bolin, 1989).

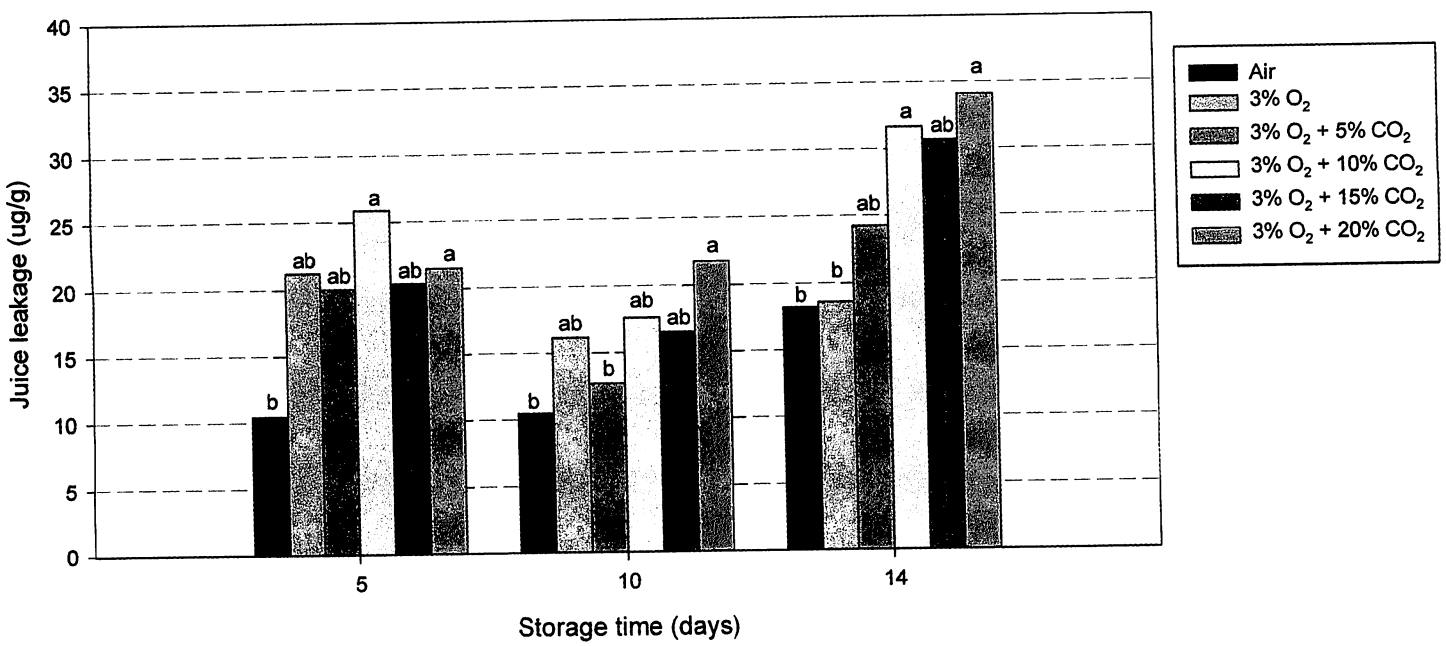

Figure 4. Juice leakage, in µg of juice/g of tissue, for fresh-cut watermelon stored under several atmospheres for 14 days at 3°C. Mean separation by Duncan's Multiple Range Test; means with the same letter for each storage day are not significantly different ($P \leq 0.05$).

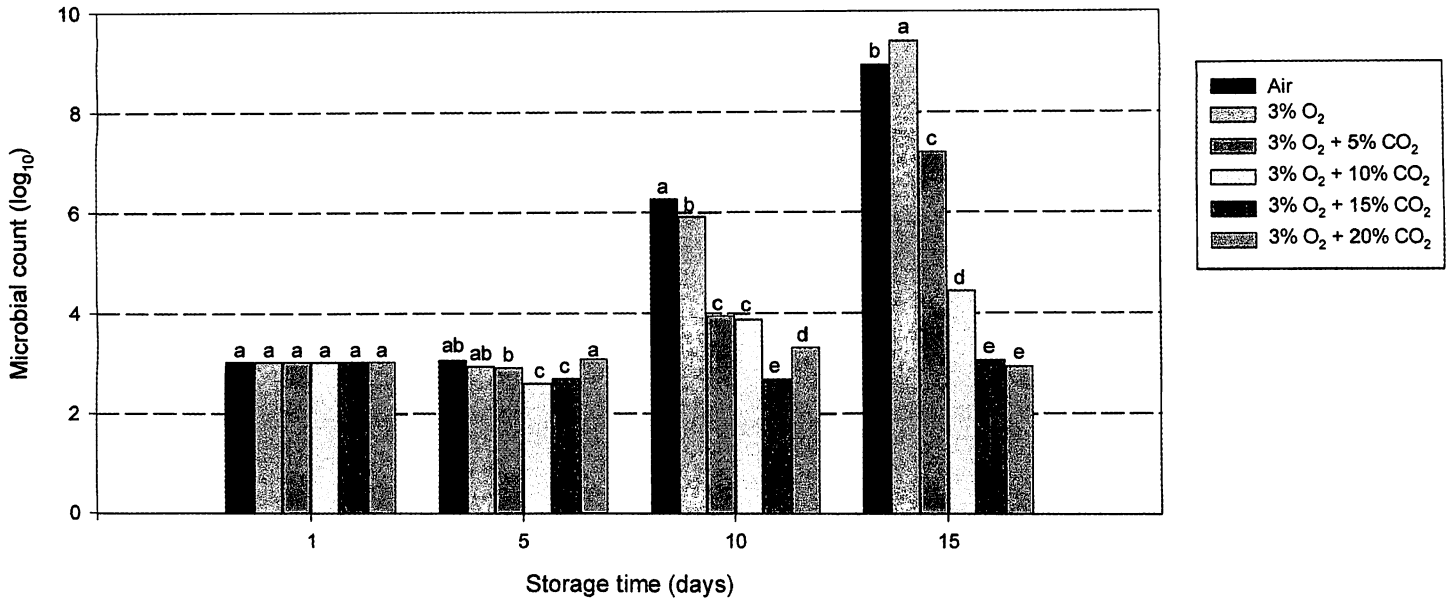

Figure 5. Microbial counts (Log_{10}) for fresh-cut watermelon stored under several atmospheres for 15 days at 3°C. Mean separation by Duncan's Multiple Range Test; means with the same letter for each storage day are not significantly different ($P \leq 0.05$).

Microbial populations increased significantly in tissues stored in air by the 5th day of storage with symptoms including cloudy juice and off-odor (Fig. 5). Bacterial population remained at initial values during the entire storage time for samples stored under 3% O_2 + 15% CO_2 and 3% O_2 + 20% CO_2. Although the 3% O_2 + 10% CO_2 regime showed a higher microbial count than the treatments with 15% and 20% CO_2, it also maintained acceptable levels of microbial load after 15 days of storage. Treatment with 3% O_2 alone did not control microbial growth, revealing the importance of elevated CO_2. This observation agrees with Sitton and Patterson's (1992) findings that high CO_2 levels are more effective than low O_2 in reducing and/or preventing decay in apples caused by several fungi. Bacteria found in the samples were not identified, however, some authors have reported that microorganisms commonly found in fresh-cut produce are pectinolytic bacteria such as *Erwinia* spp. and fluorescent *Pseudomonas* spp. These organisms are usually found in the field and/or in the microflora of raw product and are introduced during processing (King Jr. and Bolin, 1989; Nguyen-the and Carlin, 1994).

Based on these results, we conclude that 3% O_2 combined with 10%, 15% or 20% CO_2 is effective in controlling bacterial growth in fresh-cut watermelon to acceptable levels when stored at 3°C for 14 days. However, these concentrations may provoke undesirable juice leakage and visual quality such as color.

Storage under higher O_2 concentrations such as 5%, in conjunction with elevated $CO_2 \geq 10\%$ may minimize development of physiological disorders while controlling microbial growth.

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