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Research Note

Growth and Survival of *Escherichia coli* O157:H7 on Fresh-Cut Apples in Modified Atmospheres at Abusive Temperatures

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ABSTRACT

The effects of reduced-O_2 and elevated-CO_2 modified atmospheres (MAs) and abusive temperatures on the growth and survival of *E. coli* O157:H7, yeast, and molds and on changes in the visual quality of fresh-cut apples were evaluated. High-CO_2 and low-O_2 ($\geq$15% and $<$1%, respectively) atmospheres inhibited the growth of the pathogen on apple slices at 15 and 20°C. However, the population of the pathogen increased by 1 log cycle after 2 weeks of storage in air. The high-CO_2 MA resulted in the inhibition of yeast and mold growth, less browning, and better visual quality than did air and ambient-CO_2 atmospheres. The results of this study confirm that *E. coli* O157:H7 can grow on apple slices in air. These results also show that these organisms survive but are inhibited in MAs with high CO_2 levels at abusive temperatures. An MA can increase the shelf life of fresh-cut apples by improving retention of visual quality and inhibiting yeast and molds. Thus, contamination of minimally processed apples with *E. coli* O157:H7 can be a safety issue for both air- and MA-packaged cut apples.

Demand for minimally processed fresh produce has increased in the last 2 decades *(12)*. The shelf life of minimally processed products can be extended by modified atmosphere packaging (MAP) with a reduced-O_2 and/or elevated-CO_2 modified atmosphere (MA), the use of appropriate antibrowning agents, and refrigeration *(13)*. An elevated-CO_2 MA results in the inhibition of enzymatic browning and fermentative volatile production, a reduction in respiration and the ethylene production rate, and, consequently, the extension of the shelf life *(7)*.

Fresh apple products, especially juices, have been associated with outbreaks of diseases caused by *E. coli* O157:H7 *(2, 4)*. This pathogen can cause hemolytic uremic syndrome, which may result in acute renal failure in children as well as hemorrhagic colitis and thrombocytopenic purpura *(11)*. Several studies have shown that *E. coli* O157:H7 can survive and grow on fresh apple tissues stored in air *(4, 5)*. Apples can be contaminated by the pathogen through fruit flies, fecal contamination, internal contamination due to bacterial growth, water, equipment, and infected handlers *(2, 10)*. *E. coli* O157:H7 can survive at refrigerated temperatures, grow at $\geq$10°C, and survive in MAs *(8)*.

The extension of a product's shelf life theoretically could provide time for *E. coli* O157:H7 to multiply without adversely affecting the organoleptic quality of the product and therefore could increase the risk of disease, especially if the product has been temperature abused. Carbon dioxide increases the lag phase and the generation time during the logarithmic growth phase and is a major antimicrobial element of MAP at concentrations of $>$10% *(12)*. There is a lack of information on the effect of MAs on the fate of *E. coli* O157:H7 in fresh-cut products such as apples. Therefore, we investigated the effect of CO_2 in MAs on the growth and survival of *E. coli* O157:H7 and spoilage organisms in fresh-cut apples during storage at abusive temperatures.

MATERIALS AND METHODS

Media and reagents. Chlortetracycline, ampicillin, chloramphenicol, ascorbic acid, and $CaCl_2$ were purchased from Sigma (St Louis, Mo.). Citric acid was obtained from Fisher Scientific (Pittsburgh, Pa.). Peptone water, Trypticase soy agar, and Sabouraud dextrose agar were obtained from Becton Dickinson (Sparks, Md.).

Preparation of apple slices. 'Delicious' apples, harvested from Cornell University Orchards in 2000 and stored as whole apples in a controlled atmosphere (2% O_2 + 2% CO_2 [balance N_2] at 0.5°C) for 9 months, were used. Apples were hand peeled, cored, and sliced into eight equal wedges. Each wedge was further cut into smaller pieces (ca. 3 g) with a sharp knife. The pieces were dipped in antibrowning solution (1% ascorbic acid, 0.5% citric acid, and 1% $CaCl_2$) for 3 min, blot dried with sterile cheesecloth, and placed uncovered in sterile polystyrene flat-bottom 6-well tissue culture plates (well diameter 35 mm; Corning Glass Works, Corning, N.Y.).

Inoculation with *E. coli* O157:H7. Ampicillin-resistant *E. coli* O157:H7 (ATCC 43895) expressing green fluorescence (to aid in enumeration) was kindly provided by Dr. Worobo at New York State Agricultural Experiment Station, Geneva, N.Y. The strain was grown in Trypticase soy broth containing 100 μg of ampicillin per ml at 37°C with shaking in a water bath (Blue M

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TABLE 1. *Apple slice visual evaluation guide (1-to-9 scale)*[a]

Quality attribute	Characteristics for rating				
	9	7	5	3	1
Decay	No sign of decay; free from defects	Minor defects; not objectionable, little significance to consumer	Slight to moderate objectionable defects; lower limits of salability	Excessive defects, limited salability	Extremely poor; consumer reluctant to touch apple slices
Discoloration	None	Slight, not objectionable; 1–15% brown	Moderate, becoming objectionable; 16–40% brown	Severe, definitely objectionable; 41–50% brown	Extreme, >50% brown
Mold	None	Slight	Moderate	Severe	Extreme
Salability	Salable	Salable	Salable	Unsalable	Unsalable
% edible	100	90	75	50	0
Overall quality	Excellent	Good	Fair	Poor	Inedible

[a] Adapted from Diaz and Hotchkiss *(3)*.

model MSB-1122A-1, Blue Island, Ill.) for 18 to 24 h. Cell density was determined by plating on Trypticase soy agar (5×10^8 CFU/ml). This stock culture was diluted in 0.1% peptone water (10^5 CFU/ml) to prepare an inoculum. Each apple piece was inoculated with 0.1 ml of inoculum on one surface using a micropipetter. Apples used for sensory evaluations were not inoculated.

Controlled-atmosphere storage. The tissue culture plates containing the apple pieces were placed in a large plastic container (five plates per container). Two holes were fitted on opposite sides of each container, and plastic tubing was attached. Premixed gas mixtures, filtered with a 0.2-μm-pore-size filter and humidified, were continuously passed through the sealed containers at a rate of 80 ml/min during storage. The samples were stored at 15°C in 1% O_2 with 0, 15, and 30% CO_2 (balance N_2) and in air; these gas mixtures were chosen on the basis of positive effects for cut apples demonstrated in our previous work *(7)*. Cut-apple samples were evaluated every third day of the 2-week storage period. In another experiment, the samples were stored at 20°C in 21% O_2 plus 30% CO_2 (balance N_2) and in air. Samples were evaluated every other day of a 10-day storage period.

Enumeration of *E. coli* O157:H7. Apple slices from individual wells were transferred into a sterile stomacher bag (VWR Scientific, Bridgeport, N.J.) and weighed. The samples were diluted 10-fold with sterile 0.1% peptone water and homogenized for 2 min at normal speed with a stomacher (Tekmar Stomacher 400, Cincinnati, Ohio). Aliquots (1 ml) were serially diluted in 0.1% peptone water, and the dilutions were pour plated onto Trypticase soy agar in duplicate. The plates were incubated at 37°C for 48 h, and green fluorescent colonies were counted under UV light (365 nm). Three replications were carried out for each treatment.

Enumeration of yeast and molds. The homogenized and diluted samples were pour plated onto Sabouraud dextrose agar (containing 50 mg of chloroamphicenol per liter and 50 mg of chlorotetracycline per liter). The plates were incubated at 25°C for 3 to 4 days, and the total numbers of colonies were counted.

Color measurement and sensory evaluation. Apple slice color was measured for two slices from each treatment with a colorimeter (Minolta Chroma Meter Model CR-100, Minolta Camera Co. Ltd., Ramsey, N.J.). L and a values (which denote lightness-darkness and redness, respectively) were reported. Visual evaluations of apple slices were made by an untrained sensory panel ($n = 8$) by the criteria in Table 1.

Statistical analysis. Analyses of variance using one-way and general linear model procedures were performed to determine the main effects and interactions with Minitab Release 13.1 (State College, Pa.). The treatment levels were also compared by Tukey simultaneous all pairwise comparison tests.

RESULTS

MAs, compared with air, significantly inhibited the growth of *E. coli* O157:H7 on fresh apple slices ($P < 0.05$). The *E. coli* O157:H7 population remained constant at 5×10^3 CFU/g during storage in MAs with 1% O_2 plus 0, 15, and 30% CO_2, while it increased to about 10^5 CFU/g during storage in air at 15°C (Fig. 1). Carbon dioxide at 30% in an atmosphere containing 21% O_2 also inhibited the growth of *E. coli* O157:H7 at 20°C (Fig. 2). The *E. coli* O157:H7 population remained constant for 9 days of storage in 30% CO_2 plus 21% O_2, while it increased by >1 log cycle in air at 20°C (Fig. 2).

Atmospheres containing 1% O_2 plus 15 or 30% CO_2, compared with that containing air and that containing 0% CO_2 plus 1% O_2, reduced the growth of yeast and molds at 15°C ($P < 0.05$; Fig. 3). The reduction of O_2 to 1% was effective in inhibiting the growth of yeast and molds only during the first 3 days of storage at 15°C, and beyond the sixth day there was no difference between the growth of yeast and molds in 1% O_2 atmospheres and that in air. No growth of yeast and molds was detected during the first 3 days in MAs at 15°C (Fig. 3). Storage in 30% CO_2 plus 21% O_2, compared with storage in air, resulted in a decrease in the growth of yeast and molds on apple slices at 20°C ($P < 0.05$; Fig. 4).

MAs with high CO_2 and low O_2 also increased shelf life on the basis of visual evaluations (Table 2). Storage of apple slices in 15 or 30% CO_2 at 15°C resulted in higher ratings for quality attributes than did storage in CO_2-free atmospheres (all ratings < 5.0; Table 2) during 2 weeks of storage ($P < 0.05$). Low O_2 without CO_2 had only limited success in extending shelf life at 15°C; the average rating

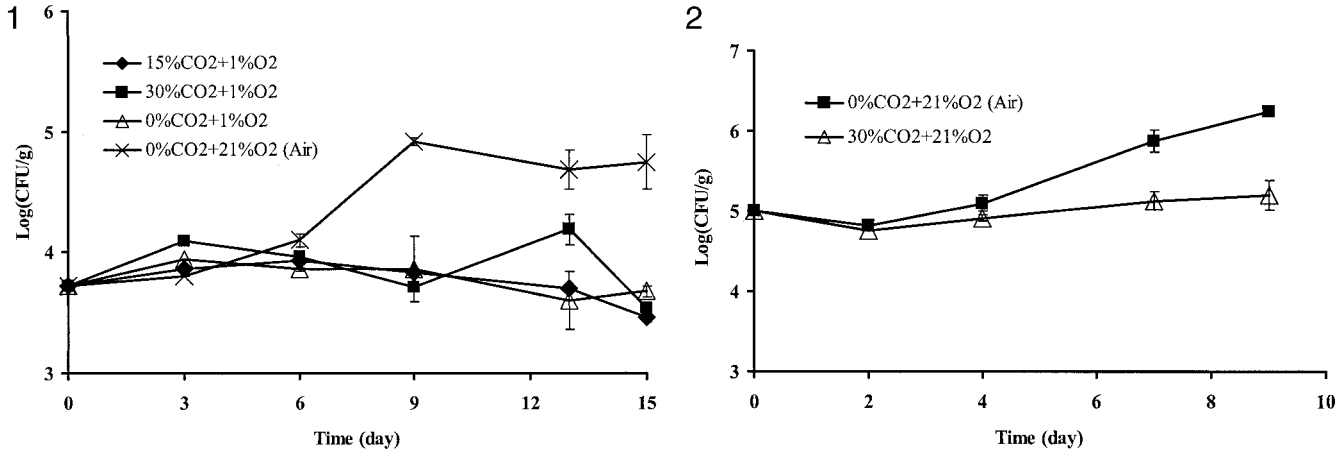

FIGURE 1. *Growth of* E. coli *O157:H7 on apple slices stored under MA conditions at 15°C. Bars represent standard errors.*
FIGURE 2. *Growth of* E. coli *O157:H7 on apple slices stored under MA conditions at 20°C. Bars represent standard errors.*

for the quality attributes fell below 5.0 beyond the sixth day of storage. CO_2 also resulted in better visual quality for apples stored in an atmosphere containing 21% O_2 at 20°C than for apples stored in air (data not shown).

MA storage also inhibited enzymatic browning. Higher L values and lower a values, indicating less browning, were obtained for apple slices stored in all three MAs than for those stored in air ($P < 0.05$; Figs. 5 and 6). The inclusion of 15 or 30% CO_2 combined with low O_2 further inhibited browning. These results were in good agreement with the visual evaluation of the slices by the sensory panel.

DISCUSSION

Researchers have shown that *E. coli* O157:H7 can grow on apple tissues in ambient atmospheres and at ambient temperatures *(4, 5, 10).* Since fresh-cut apples are packaged in MAs, we studied the fate of *E. coli* O157:H7 on fresh-cut apples in MAs to assess product safety. Our results agree with previous results *(4, 5, 10)* that indicate that the pathogen can grow on apple tissue at ambient temperatures. We extend previous findings by showing that compared with storage in ambient atmospheres, storage in MAs with high CO_2 was significantly inhibitory. Both CO_2 and O_2 in MAs had a significant effect on the growth of *E. coli* O157:H7 on apple slices. The effect of CO_2 on growth was not seen at a low O_2 concentration at 15°C, but a significant effect was detected at a higher O_2 concentration and temperature. Thus, CO_2 inhibits the growth of *E. coli* O157:H7 in fresh apple slices, although not substantially. Yeast and molds comprised the main background organisms in the tested apple slices. Carbon dioxide strongly inhibited the growth of yeast and molds on apple slices.

Visual evaluation and objective color measurement indicated that high-CO_2 MAs also improved the retention of quality. We previously demonstrated that 15 and 30% CO_2 improved the quality and inhibited the fermentation of fresh-cut apples *(7).* MAP has also been shown to inhibit polyphenol oxidase activity and to decrease the total color change of cut apples *(13).* Thus, the results of our study, along with those presented in previous literature, indicate that the shelf life of apple slices can be extended significantly by MAs containing high CO_2 levels.

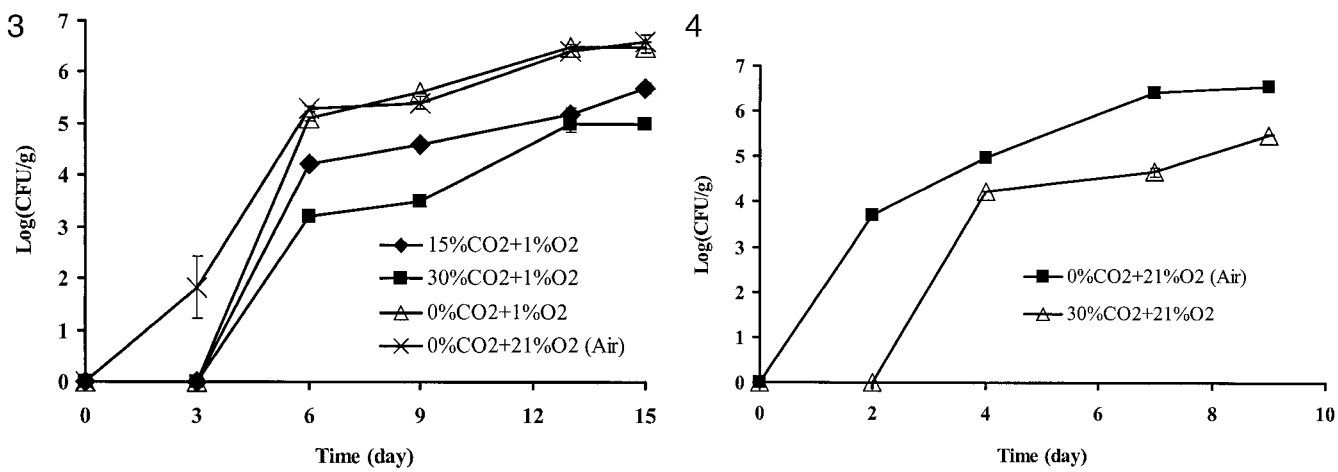

FIGURE 3. *Changes in total yeast and mold counts on apple slices stored under MA conditions at 15°C. Bars represent standard errors.*
FIGURE 4. *Changes in total yeast and mold counts on apple slices stored under MA conditions at 20°C. Bars represent standard errors.*

TABLE 2. *Visual evaluation of apple slices during storage at 15°C based on criteria in Table 1*[a]

Quality attribute	Atmosphere (O_2/CO_2)	Storage time (days)[b] 3	6	9	13	15
Decay	Air	5.7	4.8	1.8	1.2	2.0
	1/0	6.7	6.8	5.6	2.5	2.7
	1/15	6.9	6.3	7.4	6.0	7.2
	1/30	6.5	7.5	7.0	4.6	5.1
Discoloration	Air	5.9	3.8	1.1	1.1	2.1
	1/0	5.7	6.0	4.4	3.7	3.6
	1/15	7.7	5.8	6.4	5.9	7.2
	1/30	6.4	7.0	7.0	4.6	5.8
Mold	Air	9.0	9.0	7.8	3.4	3.8
	1/0	9.0	9.0	7.1	5.7	3.4
	1/15	8.8	9.0	9.0	7.7	8.8
	1/30	9.0	9.0	9.0	6.5	7.7
Salability	Air	6.0	4.0	2.1	1.1	1.9
	1/0	6.5	6.2	4.0	2.4	2.2
	1/15	7.6	5.2	5.8	4.4	7.2
	1/30	6.7	7.5	6.6	3.1	5.0
% edible	Air	7.1	3.8	1.3	1.1	1.8
	1/0	7.3	6.2	4.1	2.4	2.1
	1/15	8.1	5.5	6.3	5.6	7.0
	1/30	7.7	7.3	6.9	5.2	5.0
Overall quality	Air	6.1	4.0	2.0	1.1	2.0
	1/0	5.8	6.3	4.7	2.5	2.0
	1/15	7.0	5.7	6.6	5.8	7.1
	1/30	6.2	7.7	7.3	4.3	5.9

[a] For all attributes, both atmosphere and storage time effects were significant ($P < 0.05$). Contrasts between CO_2-containing atmospheres and CO_2-free atmospheres were also significant for all attributes ($P < 0.05$).
[b] Average of eight ratings.

The extension of shelf life by MAs could raise safety concerns *(9)*: MAs can inhibit spoilage organisms, which deter consumers from consuming the product, without affecting the viability of the pathogen. With the extension of shelf life by MAs, there will be more time for the pathogen to multiply in the product without organoleptic rejection. The extension of shelf life through the inhibition of yeast and mold growth on apple slices was observed in this work. Our results indicate that apple slices were visually acceptable for up to 13 days in MAs containing CO_2, although they contained significant numbers of *E. coli* O157:H7. The pathogen grew after 6 days in an ambient atmosphere at 15°C. The pathogen itself did not cause any apparent visual quality degradation.

Although the growth of *E.* coli O157:H7 was inhibited by MAs, its survival was not affected. Therefore, the development of a technology to eliminate this pathogen from apple slices may be required to assure safety. We have tested irradiation on apple slices and found that irradiation resulted in significant softening of apple slices at dose levels above 1 kGy *(6)*. Studies have shown that an irradiation dose of 1.8 kGy is required to achieve the required 5-log reduction in *E. coli* O157:H7 in apple juice *(1)*.

CONCLUSIONS

High CO_2 in MAs inhibited the growth of *E. coli* O157:H7, yeast, and molds on fresh-cut apples at 15 and 20°C. However, this pathogen survived well on apple slices during storage and it did not cause apparent visual defects. Storage in MAs reduced quality degradation and increased shelf life. *E. coli* O157:H7 can be a serious problem in fresh-cut apples, and MA storage alone is not sufficient to eliminate the risk posed by it. Novel processing methods such as high pressure, pulsed electric field, UV, radio frequency, irradiation, and active packaging combined with MA and refrigeration may reduce the risk; thus, more studies are needed in this area.

ACKNOWLEDGMENTS

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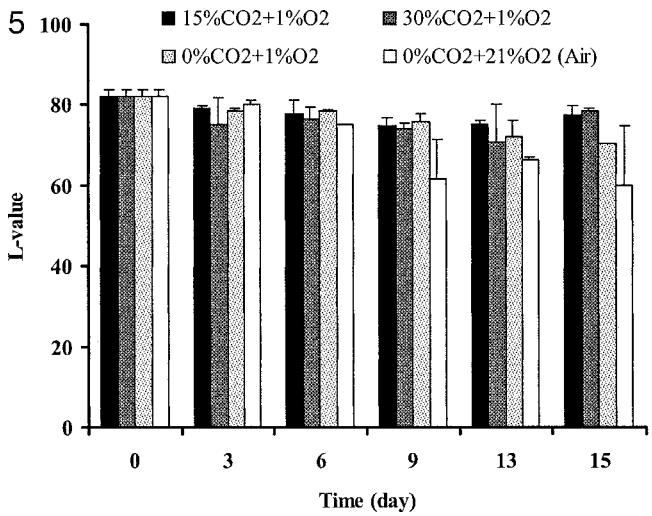

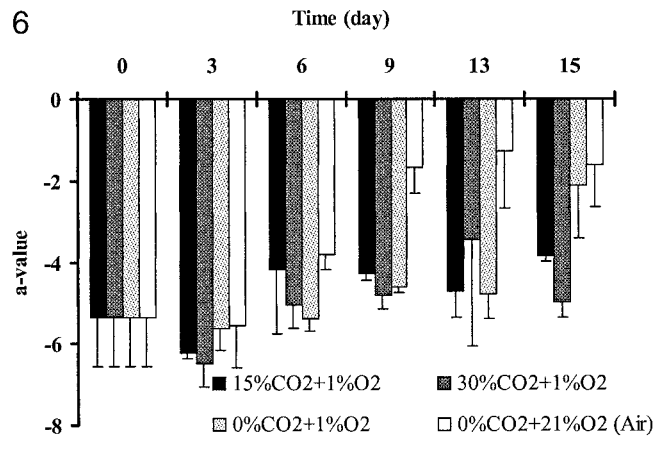

FIGURE 5. *Changes in L values for apple slices during MA storage at 15°C. Bars represent standard deviations.*
FIGURE 6. *Changes in a values for apple slices during MA storage at 15°C. Bars represent standard deviations.*

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