Shelf Life and Microbial Quality of Fresh-cut Mango Cubes Stored in High CO_2 Atmospheres

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ABSTRACT: Fresh-cut 'Carabao' and 'Nam Dokmai' mango cubes were stored in air or in high CO_2 atmospheres (3%, 5%, and 10%) at 5 °C and 13 °C. Freshly sliced 'Carabao' mango cubes had a lower respiration rate and total bacterial count and higher L-ascorbic acid content and firmness than 'Nam Dokmai' mango cubes. The shelf life of fresh-cut mango, based on browning discoloration and water-soaked appearance, was 6 d at 5 °C and 4 d at 13 °C for 'Carabao' and 2 d at 5 °C and less than 1 d at 13 °C for 'Nam Dokmai'. High CO_2 atmospheres retarded the development of water-soaked 'Carabao' cubes at 5 °C and 13 °C and 'Nam Dokmai' cubes at 5 °C. Texture of 'Carabao' cubes was enhanced by high CO_2, but ethanol and L-ascorbic acid contents were not affected at 5 °C and 13 °C. Total bacterial count was lower in 'Carabao' cubes than in 'Nam Dokmai' cubes during storage at both temperatures, and a 10% CO_2 only reduced the bacterial count on 'Carabao' and 'Nam Dokmai' cubes stored at 13 °C. Bacterial flora in 'Nam Dokmai' mango cubes consisted mostly of Gram-negative rods assigned primarily to phytopathogenic bacteria such as *Pantoea agglomerans* and *Burkholderia cepacia*. The genera of bacteria isolated from cubes stored in 10% CO_2 were similar to those from cubes on the initial day.
Keywords: fresh-cut, mango, shelf life, bacterial flora, controlled atmosphere

Introduction

The shelf life of fresh-cut mangoes is affected by cultivar, fruit ripeness, and storage temperature. Rattanapanone and others (2001) reported that the marketable period of fresh-cut 'Tommy Atkins' and 'Kent' mango cubes was 3 to 5 d at 10 °C and 5 to 8 d at 5 °C. Allong and others (2000) showed that firm-ripe 'Graham' and half-ripe 'Julie' mango slices had a shelf life of 8 d at 5 °C and 10 °C and half-ripe 'Graham' and firm-ripe 'Julie' mango slices had a shelf-life of 4 d. They also found that mature-green fruit was not recommended for fresh-cut due to lack of sensory quality and short shelf life. We reported that the shelf life of 'Carabao' mango cubes was 4 to 6 d at 5 °C and 3 to 4 d at 13 °C and was affected by maturity (Izumi and others 2003), whereas the shelf life of 'Nam Dokmai' mango cubes was only 3 d at 1 °C, 2 d at 5 °C, and less than 1 d at 13 °C (Poubol and others 2005).

Controlled atmosphere (CA) storage and modified atmosphere packaging (MAP), which reduced O_2 levels and/or increased CO_2 levels, have been shown to extend the shelf life of fresh-cut mangoes. Low O_2 atmospheres (0.5%, 1%, and 2%) were beneficial in maintaining the quality of fresh-cut 'Carabao' mango cubes stored at 5 °C and 13 °C (Izumi and others 2003) and extending the shelf life of 'Nam Dokmai' mango cubes by 1 d at 1 °C, 5 °C, and 13 °C by retarding the brown discoloration and water-soaked appearance (Poubol and others 2005). The shelf life of fresh-cut 'Tommy Atkins' and 'Kent' mango cubes was extended 1 to 2 d by 4% O_2 + 10% CO_2 or 2% O_2 + 10% CO_2 atmospheres, which retarded browning, water-soaked appearance, and microbial growth (Rattanapanone and others 2001). Fresh-cut 'Keitt' mango could be held in active MAP having a gas mixture of 4% O_2, 10% CO_2 and 86% N_2 for 25 d at 5 °C, under

which the microbial growth was inhibited by 10% CO_2 and fruits continued aerobic respiration under the combination of a low O_2 and high CO_2 atmosphere (Martínez-Ferrer and others 2002). With other fresh-cut fruits, elevated CO_2 reduced respiration rates, ethylene production, and fermentative metabolite production of fresh-cut apple slices at 5 °C (Gunes and others 2001), maintained high firmness, good visual quality, low ethylene production, and low accumulation of fermentative products of fresh-cut kiwifruit slices at 0 °C (Agar and others 1999) and had an inhibitory effect on accumulation of fermentation products of fresh-cut peach and nectarine at 10 °C (Gorny and others 1999). However, high CO_2 atmospheres of 10% to 20% accelerated tissue browning and necrosis of fresh-cut pear slices at 5 °C (Gorny and others 2002), degradation of vitamin C in fresh-cut kiwifruit slices at 2 °C (Agar and others 1999), and reduction of retinol equivalent, calculated from carotenoid content, in fresh-cut peach slices at 5 °C (Wright and Kader 1997). Thus, the quality responses of fresh-cut mango to elevated CO_2 need to be investigated to determine the optimum ranges of CO_2 acceptable in MAP.

It is also important to consider microbial safety of fresh-cut mangoes during storage. Recent research indicated that a recent outbreak of illness due to *Salmonella* serotype Newport was epidemiologically associated with consumption of mangoes (Sivapalasingam and others 2003). The hot and cold water treatments for fruit fly disinfestations were the most likely source of contamination, in which entry of *Salmonella* into the stem-end segment (83%) was significantly higher than into the middle-side (19%) or blossom-end (9%) segments (Penteado and others 2004).

In this study, we initially determined the physiology and quality of freshly sliced cubes from partially ripe 'Carabao' and 'Nam Dokmai' mangoes. Subsequently, we determined the effects of high CO_2 atmospheres (3%, 5%, and 10%) on physiology, quality, and microbial quality and safety of fresh-cut 'Carabao' and 'Nam Dokmai' mangoes during storage at 5 °C and 13 °C.

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Materials and Methods

Plant materials

'Carabao' and 'Nam Dokmai' mangoes (*Mangifera indica* L.) were imported from Philippine and Thailand, respectively. Fruit at partially ripe stage (50% to 60% skin yellowing) were selected, washed thoroughly with tap water, and dipped for 10 min in chlorinated water (50 ppm available chlorine). After peeling, the flesh was removed from the seed, sliced, and cut into 2 cm^3 cubes.

High CO_2 controlled atmosphere storage

A 100-g sample (about 12 cubes) was placed in a 500-mL plastic container containing 5 mL of distilled water in a beaker to maintain high relative humidity. Three replicated samples were stored under a continuous flow of air or high CO_2 atmospheres (3%, 5%, and 10%) with the balance being air, at a flow rate of 10 mL/min at 5 °C and 20 mL/min and 13 °C.

Physiology and quality evaluation

Evaluations of brown discoloration and water-soaked appearance were made for each cube as the visual quality and were expressed as the percentage of the total number of cubes with defects in each container. The cubes were considered to be at the threshold or lower limit of marketability when 20% of the cubes had browning or 60% had water-soaked appearance.

The CO_2 contents of inlet and outlet streams of each container were monitored during storage with CO_2 analyzer (Model CD-3A, Ametek; Pittsburgh, Pa., U.S.A.) to calculate the respiration rate. Ethanol content in the flesh was determined using a gas chromatograph (GC) equipped with a flame ionization detector at 150 °C and a glass column (3 mm × 1.5 m) containing 5% Tween 20 on 60/80 Celite 545 as stationary phase at 60 °C (Izumi and others 2003).

The color of 5 cubes from each container was measured with a Handy Colorimeter (Model NR-3000, Nippon Denshoku, Tokyo, Japan). The L^* value was used as an indicator of cut surface browning intensity (Sapers and Douglas 1987). Texture of the cubes was measured by the force required to shear a cube with a cutter blade using an EZ test (Model AR-228, Shimadzu, Kyoto, Japan) and was expressed as a maximum shear force (N). L-Ascorbic acid (AsA) content of the cubes was determined using high-performance liquid chromatography (Model LC-10AD; Shimadzu, Kyoto, Japan) equipped with PLRP-S 100A column (4.66 mm × 25 cm, 5 μm; Polymer Laboratories, Amherst, Mass., U.S.A.) and electrochemical detector (Model ECD 300; Eicom, Kyoto, Japan) as previously described (Izumi and Watada 1995).

Bacterial count

Total bacterial count in mango cubes was performed as described by Izumi (1999). A 10-g sample was macerated in 90 mL of sterile saline solution (0.85% NaCl water) in a sterile stomacher bag using an Elmex stomacher (Pro-media SH-001; Eiken Kizai, Tokyo, Japan) for 4 min at room temperature. The serial dilutions of this solution were made in sterile saline solution and poured in duplicate Standard Method Agar (SMA; Nissui Pharmaceutical, Tokyo, Japan) plates, which were incubated at 37 °C for 48 h for the enumeration of total bacteria. Total bacterial count was expressed as log_{10} colony-forming units (CFU)/g.

Bacterial isolation

The Gram-negative and Gram-positive bacteria were aseptically isolated from the homogenates of mango samples using Crystal Violet Triphenyl-Tetrazolium-Chloride (TTC) Agar (CVT; Nissui Pharmaceutical, Tokyo, Japan) incubated at 25 °C for 48 to 72 h and SMA (Nissui Pharmaceutical, Tokyo, Japan) with 0.3% β-phenylethanol incubated at 30 °C for 48 to 72 h, respectively. Pure cultures of bacteria

Table 1 — Physiology and microbial quality of fresh-cut 'Carabao' and 'Nam Dokmai' mango after slicing

Physiology and microbial quality	Cultivars		
	'Carabao'	'Nam Dokmai'	
Respiration rate (mg/kg•h)	10.2[a]	13.2[a]	**
	39.3[b]	41.8[b]	*
L-ascorbic acid (mg/100 g FW)	35.6	22.9	**
Firmness (N)	5.2	1.3	**
Total microbial count (Log_{10}CFU/g)	<2.4	4.1	**

[a]After storage for 1 d at 5 °C.
[b]After storage for 1 d at 13 °C.
*,**Significant at $P \leq 0.05$ or 0.01, respectively, between pared 'Carabao' and 'Nam Dokmai' cultivars.

were subjected to an extensive phenotypic investigation, including cell morphology observed by microscopy and Gram stain (Cappuccino and Sherman 1992). Seventy-nine bacterial isolates were selected from different-appearing colonies by a stereoscopic observation.

Bacterial identification by MicroSeq™ sequence analysis of 16S rDNA

The MicroSeq™ System (PE Applied Biosystems, Foster City, Calif., U.S.A.) consisted of a polymerase chain reaction (PCR) and cycle sequencing module, bacterial identification and analysis software, and a 16S rDNA sequence database library as previously described (Tang and others 1998). Bacterial genomic DNA was prepared from bacterial isolates using the DNeasy® Tissue Kit (Qiagen Sciences, Valencia, Calif., Md, U.S.A.). The 1st 527 bp of the 16S rRNA gene (Brosius and others 1978) were amplified from the bacterial genomic DNA by PCR using a PCR Master Mix including AmpliTaq® Gold DNA Polymerase and 0005F (5′-TGG AGA GTT TGA TCC TGG CTC AG-3′) and 0531R (5′-TAC CGC GGC TGC TGG CAC-3′) primers (TaKaRa, Shiga, Japan).

The PCR product was purified with ExoSAP-IT™ (U.S. Biochemical Corp., Cleveland, Ohio, U.S.A.) including Exonuclease I and Shrimp Alkaline Phosphatase in buffer before the sequencing reaction. The MicroSeq system was used for sequencing of the PCR products. The sequencing reaction consisted of Complete Sequencing Mix (Forward or Reverse Sequencing Mix; with AmpliTaq® DNA Polymerase, FS and dRhodamine Dye Terminators), purified amplified product, and sterile MQ water. The sequences were determined by using electrophoresis with the ABI PRISM™ 310 Genetic Analyzer (PE Applied Biosystems). The sequencing data was analyzed using the MicroSeq™ Microbial Identification and Analysis Software (MicroSeq™ Analysis Software v. 1.40 and MicroSeq™ 16S rDNA Sequence Databases v.1.01). The database comparison, using the Basic Local Alignment Search Tool (BLAST) and Full Alignment Tool of MicroSeq, presented a list of the closest matches with a distance score. A cutoff of the lowest distance score was chosen for species identity.

Statistical analysis

Data were subjected to analysis of variance and the Duncan's Multiple Range test, and the standard error of each mean is presented in the figures.

Results and Discussion

Comparison of fresh-cut 'Carabao' and 'Nam Dokmai' mango cubes in physiology and microbial quality

'Carabao' mango cubes just after slicing had a lower respiration rate and total bacterial count and a higher L-ascorbic acid content and firmness than 'Nam Dokmai' mango cubes (Table 1). The difference in physiology and quality between 2 mango cultivars just

after slicing probably relates to the difference in the shelf life of the two-cultivar mango cubes, which were previously reported separately (Izumi and others 2003; Poubol and others 2005). This fact may indicate that 'Carabao' cultivar is more suitable than 'Nam Dokmai' cultivar for fresh-cut mango.

Effects of high CO_2 atmospheres on physiology and quality

The percentage of brown discoloration in 'Carabao' mango cubes was below the maximum level (20%) to be unmarketable during storage at 5 °C, whereas 'Nam Dokmai' mango cubes developed brown discoloration to an unmarketable level with storage at 5 °C, and no major differences were found between samples in air and high CO_2 atmospheres (Figure 1). This observation was confirmed by L^* values on cut surface of cubes (data not shown). Water-soaked appearance developed on 'Carabao' and 'Nam Dokmai' mango cubes stored at 5 °C, with the development being greater in air than in high CO_2 atmospheres. The percentage of water-soaked appearance on cubes in high CO_2 was almost below the maximum level (60%) to be unmarketable during storage at 5 °C, whereas at 13 °C, 'Carabao' cubes stored in air developed brown discoloration and water-soaked appearance up to the threshold of marketability for browning (20%) and water-soaked appearance (60%), respectively, by the end of storage (Figure 2). High CO_2 atmospheres inhibited the development of water-soaked appearance in 'Carabao' cubes. All 'Nam Dokmai' cubes at 13 °C began to develop browning and water-soaked appearance very rapidly and became unmarketable on day 1. A high CO_2 has been reported to reduce enzymatic browning in lettuce tissue by inhibition of phenolic production and polyphenol oxidase activity (Siriphanich and Kader 1985), but the inhibitory effect of high CO_2 on discoloration of mango cubes was limited, as noted with apple slices (Gunes and others 2001). Although translucency of the water-soaked area has been considered a chilling injury symptom with zucchini squash slices (Izumi and Watada 1995) and tomato slices (Gil and others 2002), the water-soaked appearance on mango cubes occurred at a chilling temperature of 5 °C and at a nonchilling temperature of 13 °C. Nevertheless, the high CO_2 atmospheres were helpful in retarding the development of water-soaked appearance in mango cubes.

Respiration rates of 'Carabao' and 'Nam Dokmai' mango cubes held in air or high CO_2 were relatively constant during storage at 5 °C and 13 °C (Figure 3). Respiration rates of cubes in 10% CO_2 at 5 °C seemed to be higher than those in air, but no differences occurred between cubes in air and high CO_2 at 13 °C. Ethanol content was not detectable from any cubes (data not shown). This result suggested that respiration may not have changed from the aerobic to anaerobic pathway as noted with some fruits stored in low O_2 atmospheres (Ke and others 1991).

Texture based on shear force of 'Carabao' cubes stored at 5 °C and 13 °C was enhanced by high CO_2, whereas that of 'Nam Dokmai' cubes in air and high CO_2 remained unchanged during storage at both temperatures (Figure 4; data shown only for 5 °C). Effects of high CO_2 on firmness vary among fresh-cut products. With kiwifruit slices, treatment with 10% CO_2 atmosphere resulted in higher firmness than air-control slices (Agar and others 1999), but high CO_2 of 5% to 20% did not affect the texture of pear slices stored at 5 °C (Gorny and others 2002) and peach slices and persimmon slices stored at 5 °C (Wright and Kader 1997). Harker and others (2000) speculated that the mechanism for CO_2-induced firmness enhancement in strawberry was due to changes in the pH of the apoplast, which may promote the precipitation of soluble pectins and thus enhance cell-to-cell bonding in strawberry fruit.

L-Ascorbic acid content of all cubes decreased during storage except for 'Nam Dokmai' mango cubes stored at 13 °C, where the content did not change (Figure 4; data shown only for 5 °C). High CO_2 atmospheres had a greater influence on degradation of L-ascorbic acid in fresh-cut kiwifruit slices (Agar and others 1999) and some berry fruits (Agar and others 1997). However, it is found that high CO_2 of 3% to 10% did not have detrimental effects on the retention of L-ascorbic acid content of fresh-cut mango.

Effects of high CO_2 atmospheres on microbial quality and safety

Total bacterial count on the cubes on the initial day was below the detection level (2.4 $\log_{10}$CFU/g) in 'Carabao' and ranged from 2.9 to 5.3 $\log_{10}$CFU/g in 'Nam Dokmai' (Table 2), which mainly contributed to the difference in shelf life between 2 cultivars as previously noted with fresh-cut 'Carabao' mango (Izumi and others
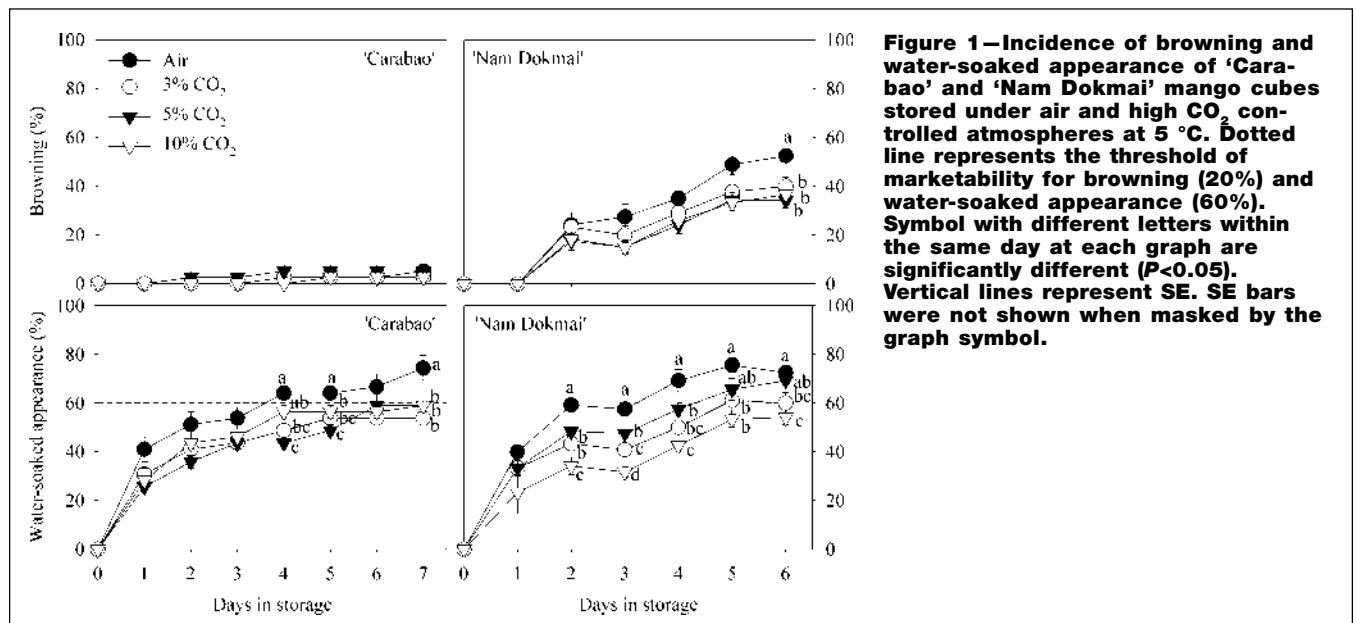

Figure 1—Incidence of browning and water-soaked appearance of 'Carabao' and 'Nam Dokmai' mango cubes stored under air and high CO_2 controlled atmospheres at 5 °C. Dotted line represents the threshold of marketability for browning (20%) and water-soaked appearance (60%). Symbol with different letters within the same day at each graph are significantly different (*P*<0.05). Vertical lines represent SE. SE bars were not shown when masked by the graph symbol.

2003) and 'Nam Dokmai' mango (Poubol and others 2005). The counts were similar and did not increase with cubes in air and high CO_2 atmospheres at 5 °C, whereas at 13 °C, the population was higher on the last day than on the initial day, except for the cubes of both cultivars held in 10% CO_2. This indicated that high CO_2 of 10% helped in reducing bacterial counts on 'Carabao' and 'Nam Dokmai' mango cubes when stored at high temperatures, which is conducive for microbial proliferation.

The total microbial counts on fresh-cut mango vary widely among cultivars and maturity, depending on acid content and pH that affected the microbial growth. Counts of 7.2 $log_{10}CFU/g$ were found in 'Keitt' mango cubes (Martínez-Ferrer and others 2002), 2.7 to 3.7 $log_{10}CFU/g$ in 'Julie' mango slices, 2.7 to 4.1 $log_{10}CFU/g$ in 'Graham' mango slices (Allong and others 2000), 3.8 to 5.2 $log_{10}CFU/g$ in 'Tommy Atkins' mango cubes, and 2.7 to 3.8 $log_{10}CFU/g$ in 'Kent' mango cubes (Rattanapanone and others 2001). CA of 2% O_2 and 10% CO_2 reduced total bacterial and yeast/mold count of 'Tommy Atkins' and 'Kent' mango cubes stored at 10 °C (Rattanapanone and others 2001); active MAP of 4% O_2 and 10% CO_2 also reduced bacterial and yeast/mold count of 'Keitt' mango cubes at 5 °C (Martínez-Ferrer and others 2002). Because low O_2 atmospheres (0.5%, 1%, and 2%) alone did not affect the
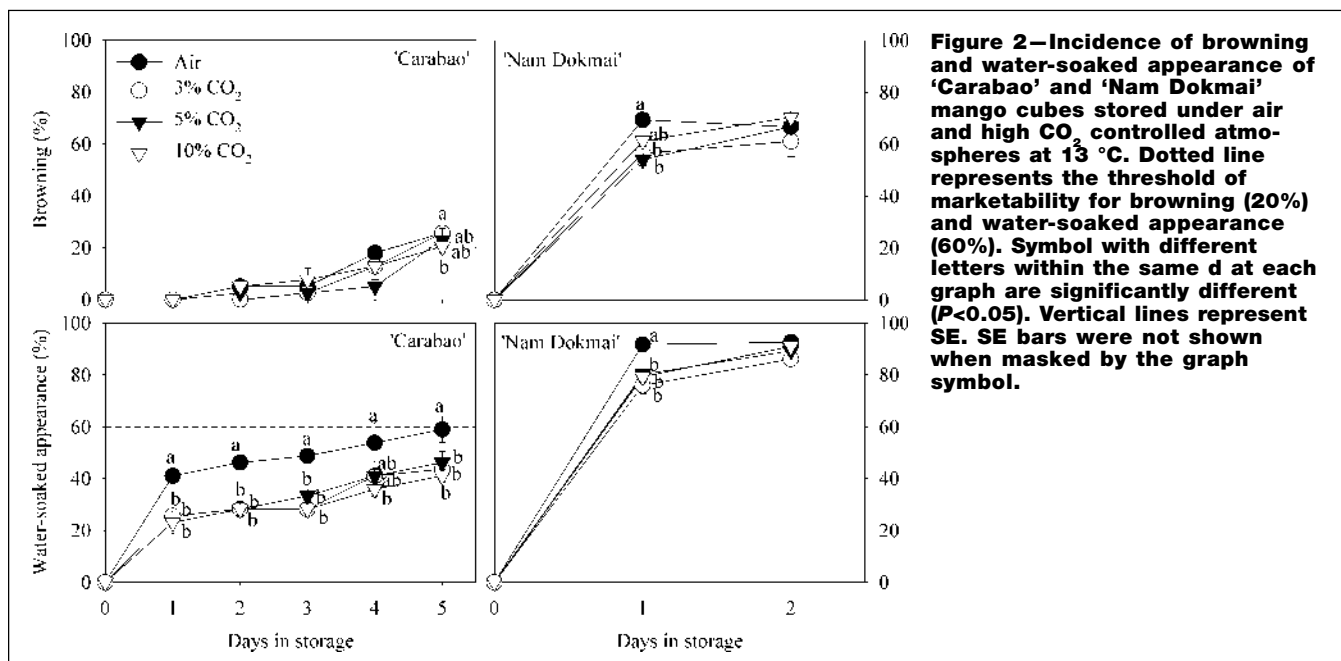

Figure 2—Incidence of browning and water-soaked appearance of 'Carabao' and 'Nam Dokmai' mango cubes stored under air and high CO_2 controlled atmospheres at 13 °C. Dotted line represents the threshold of marketability for browning (20%) and water-soaked appearance (60%). Symbol with different letters within the same d at each graph are significantly different ($P<0.05$). Vertical lines represent SE. SE bars were not shown when masked by the graph symbol.
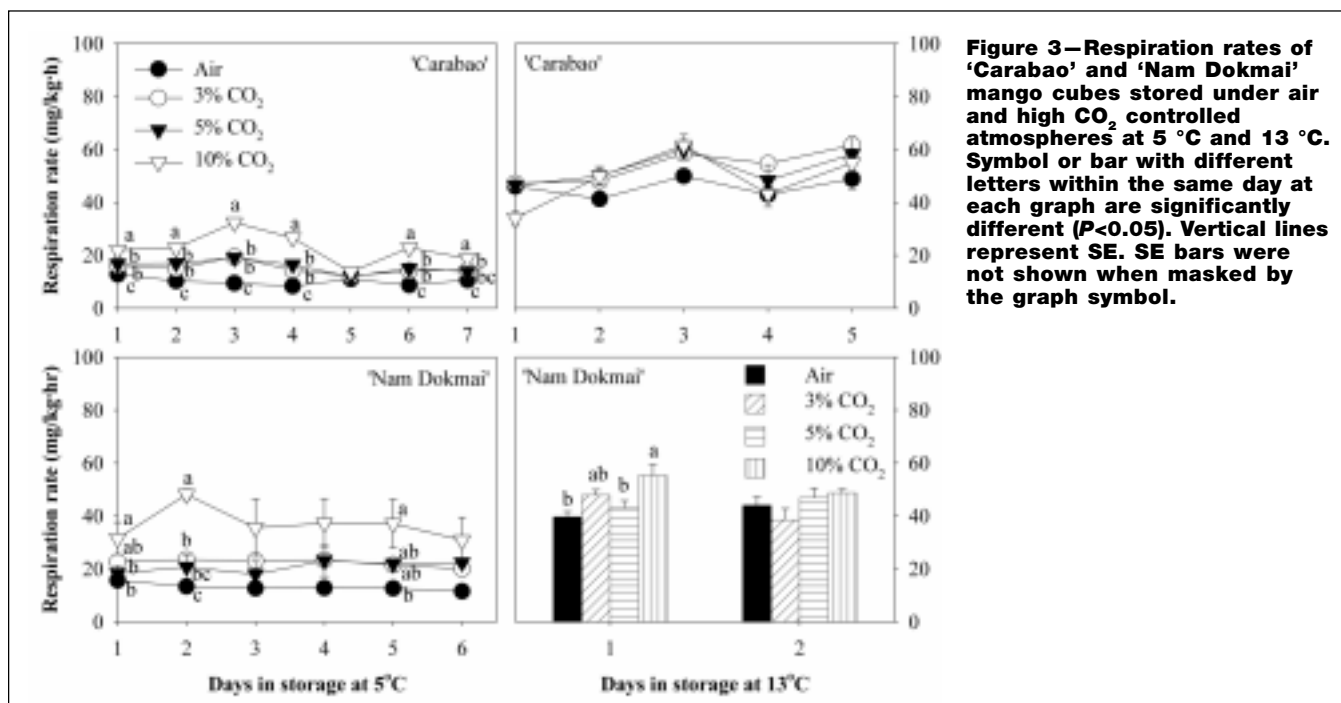

Figure 3—Respiration rates of 'Carabao' and 'Nam Dokmai' mango cubes stored under air and high CO_2 controlled atmospheres at 5 °C and 13 °C. Symbol or bar with different letters within the same day at each graph are significantly different ($P<0.05$). Vertical lines represent SE. SE bars were not shown when masked by the graph symbol.

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counts of total bacteria and lactic acid bacteria on 'Nam Dokmai' mango cubes stored at 1 °C, 5 °C, and 13 °C (Poubol and others 2005), high CO_2 seems to be beneficial on controlling microbial growth. A model system to investigate the growth of pure bacterial cultures on a solid surface under different gas phase conditions revealed that the O_2 concentrations did not affect growth of vegetable-associated microorganisms, whereas high CO_2 reduced growth in most cases (Bennik and others 1995; Amanatidou and others 1999). Inhibition by CO_2 may result from dissolved CO_2 in the aqueous phase of food products, which causes a decrease of the intracellular pH, inhibits enzymatically catalyzed reactions and enzyme synthesis, and interacts with the cell membrane (Molin 2000). However, because increases in CO_2 concentration >30% to 40% showed extensive spoilage of fresh-cut endive (Carlin and others 1996) and induced lactic acid fermentation of fresh-cut carrots (Carlin and others 1990), the limit of high CO_2 concentration should be clarified with each fresh-cut product.

The bacteria isolated from 'Nam Dokmai' mango cubes were predominantly Gram-negative rod-forms, in which about 60% of isolates were Enterobacteriaceae including the genera *Klebsiella* and *Pantoea* (Table 3). Phytopathogenic bacteria that cause rot in vegetables such as *Pantoea agglomerans* (synonymous with *Erwinia herbicola* and *Enterobacter agglomerans*) and *Burkholderia cepacia* (synonymous with *Pseudomonas cepacia*) were also isolated frequently. The most common Gram-positive bacteria found were the genus *Curtobacterium*. All bacteria isolated from mango cubes are found frequently in soil, water, air, and plants (Bartz and Wei 2003) and were similar to those found on some fresh marketed vegetables (Izumi and others 2004). Although opportunistic bacteria such as *Burkholderia cepacia* were identified, no human pathogens were detected in any of the samples in our method of bacterial isolation and identification. In stored cubes, the diversity of the microbial flora was less in the cubes in 10% CO_2 than in other samples. The genera of bacteria isolated from cubes stored in 10% CO_2 were similar to those from cubes on initial day. High CO_2 is also expected to inhibit growth of not only spoilage bacteria but also foodborne pathogenic bacteria (Bennik and others 1995; Amanatidou and others 1999; Kimura and others 1999).

Table 2—Total bacterial count of fresh-cut 'Carabao' and 'Nam Dokmai' mango cubes stored under air and high CO_2 controlled atmospheres at 5 °C and 13 °C[a]

Temp. (°C)	Days in storage	Treatment	Log$_{10}$CFU/g	
			'Carabao'	'Nam Dokmai'
5	0		<2.4	2.9
	7 ('Carabao') or	Air	<2.4	<2.4
	6 ('Nam Dokmai')	3% CO_2	<2.4	2.6
		5% CO_2	<2.4	<2.4
		10% CO_2	ND[b]	<2.4
13	0		<2.4	5.3
	5 ('Carabao') or	Air	3.2a	6.5a
	2 ('Nam Dokmai')	3% CO_2	3.8a	6.4a
		5% CO_2	2.9a	6.3a
		10% CO_2	< 2.4b	3.7b

[a]Different letters within each cultivar in the same day are significantly different ($P \le 0.05$).
[b]Not detectable.

Conclusions

Development of browning and water-soaked appearance was found to limit the shelf life and visual quality of fresh-cut mango. When the threshold of consumer acceptability was assumed to be 20% for browning and 60% for water-soaked appearance, the shelf life of fresh-cut mango was 6 d at 5 °C and 4 d at 13 °C for 'Carabao' and 2 d at 5 °C and less than 1 d at 13 °C for 'Nam Dokmai'. High CO_2 atmospheres of 3%, 5%, and 10% extended the shelf life by 3 d at 5 °C and 1 d at 13 °C for 'Carabao' and 1 d at 5 °C for 'Nam Dokmai' without deleterious effects on respiration rates, ethanol content, texture, L-ascorbic acid content, and bacterial count. A 10% CO_2 atmosphere was recommended to reduce bacterial population and bacterial diversity in microbial flora when storage temperature abuse occurred.

Acknowledgments

Figure 4—Texture based on shear force and L-ascorbic acid content of 'Carabao' and 'Nam Dokmai' mango cubes stored under air and high CO_2 controlled atmospheres at 5 °C. Bar with different letters within the same day are significantly different ($P<0.05$). Vertical lines represent SE.
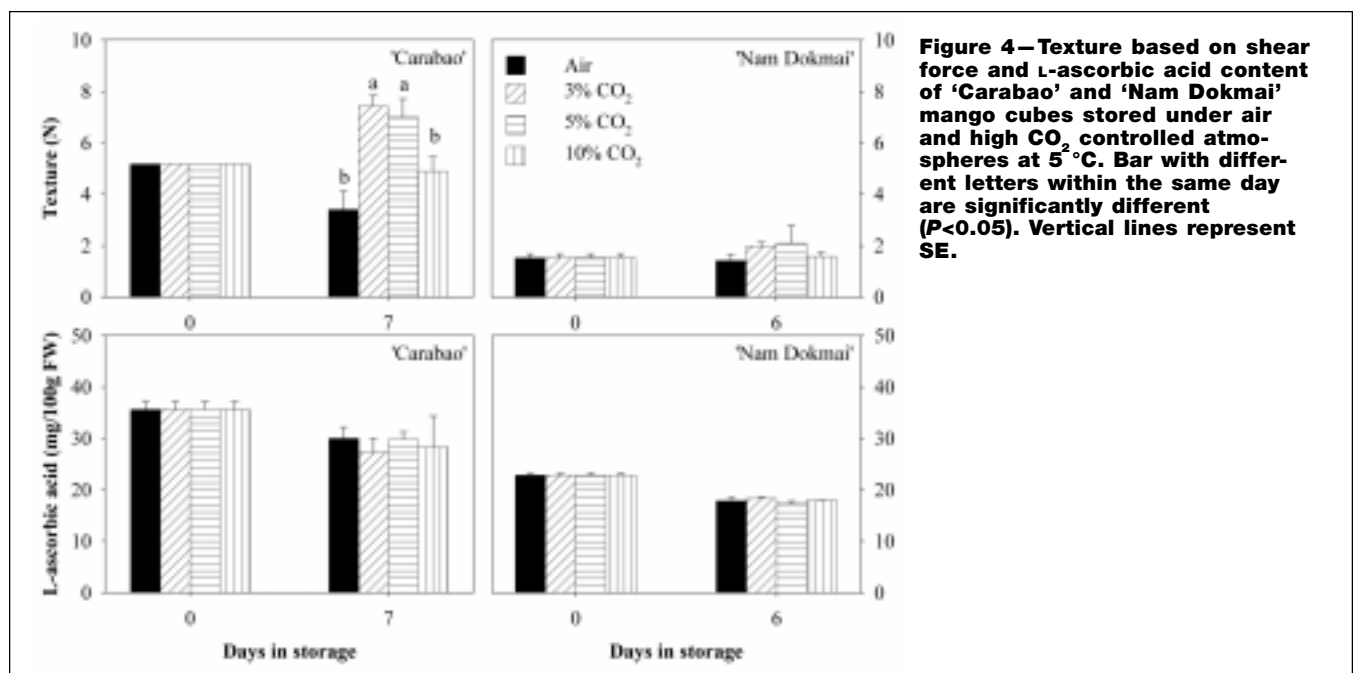
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Table 3—Bacteria isolated from fresh-cut 'Nam Dokmai' mango stored under air and high CO$_2$ controlled atmospheres at 5 °C and 13 °C

Days in storage	Temp. (°C)	Treatment	Gram type	Bacteria	
				Genus	Species
0	5		Gram-negative	*Burkholderia*	*cepacia*
				Klebsiella	*ornithinolytica*
				Klebsiella	*pneumoniae rhinoscleromatis*
6		Air	Gram-negative	*Burkholderia*	*cepacia*
				Klebsiella	*ornithinolytica*
				Pantoea	*agglomerans*
			Gram-positive	*Kocuria*	*rosea*
		3% CO$_2$	Gram-negative	*Burkholderia*	*cepacia*
				Pantoea	*agglomerans*
			Gram-positive	*Tsukamurella*	*inchonensis*
		5% CO$_2$	Gram-negative	*Burkholderia*	*cepacia*
				Frateuria	*aurantia*
				Pantoea	*agglomerans*
			Gram-positive	*Curtobacterium*	*luteum*
		10% CO$_2$	Gram-negative	*Burkholderia*	*cepacia*
				Klebsiella	*pneumoniae pneumoniae*
				Klebsiella	*pneumoniae rhinoscleromatis*
0	13		Gram-negative	*Pantoea*	*agglomerans*
				Pantoea	*stewartii indologenes*
2		Air	Gram-negative	*Pantoea*	*agglomerans*
			Gram-positive	*Curtobacterium*	*citreum*
		3% CO$_2$	Gram-negative	*Flavimonas*	*oryzihabitans*
				Pantoea	*agglomerans*
				Pantoea	*dispersa*
		5% CO$_2$	Gram-negative	*Pantoea*	*agglomerans*
			Gram-positive	*Curtobacterium*	*citreum*
		10% CO$_2$	Gram-negative	*Pantoea*	*agglomerans*
				Pantoea	*stewartii indologenes*

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