

ORIGINAL ARTICLE

Effects of pure oxygen and reduced oxygen modified atmosphere packaging on the quality and microbial characteristics of fresh-cut pineapple

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Abstract – Introduction. Modified atmosphere packaging (MAP) is a preservation technique currently used by the fresh-cut fruit industry. Fruit quality may vary according to the concentration of oxygen (O₂) in the packaging. However, there is no published research on the effects of a pure O₂ modified atmosphere in the packaging of fresh-cut pineapple. There are also no comparative studies of the differences between pure O₂ and conventional low O₂ MAP on the quality of fresh-cut pineapple. **Materials and methods.** Pineapple slices were sealed with a tray sealer using a polyethylene (PE) / polypropylene (PP) composite film and one of the following atmosphere treatments: (4% O₂ + 5% CO₂), (100% O₂), and ambient air (control). We evaluated the effects on quality and microbial spoilage of fresh-cut pineapple. **Results and discussion.** Both modified atmosphere treatments delayed decreases in firmness, soluble solid contents (SSC), reducing sugar, and ascorbic acid. Pineapple slices packaged in pure O₂ contained lower amounts of sugar and ascorbic acid and displayed more browning than the slices in the low O₂ concentration. Additionally, both modified atmosphere treatments strongly delayed the growth of microorganisms. Aerobic bacteria, yeast and mold levels in pineapple slices packaged in pure O₂ were higher than those packaged with the low O₂ atmosphere during long-term storage. **Conclusion.** Modified atmosphere packaging using low O₂ concentration (4% O₂ + 5% CO₂) was better able to maintain the quality of fresh-cut pineapple than packaging with pure O₂ atmosphere.

Keywords: pineapple / *Ananas comosus* / fresh-cut produce / controlled atmosphere packaging / food safety

Résumé – Effets du conditionnement sous atmosphère contrôlée, appauvrie ou enrichie en oxygène, sur la qualité et l'évolution microbienne de l'ananas fraîchement découpé. **Introduction.** Le conditionnement sous atmosphère modifiée (MAP) est une technique de conservation actuellement utilisée par l'industrie des fruits frais découpés. La qualité des fruits peut varier en fonction de la concentration en oxygène (O₂) dans l'emballage. Pourtant, rares sont les recherches publiées sur l'effet d'une atmosphère en O₂ pur dans l'emballage d'ananas fraîchement découpé. De même, il n'existe aucune étude comparative des différences entre l'atmosphère en O₂ pur et l'atmosphère classique basse en O₂ sur la qualité de l'ananas fraîchement découpé. **Matériel et méthodes.** Des tranches d'ananas ont été scellées sous film plastique composite en polyéthylène (PE) / polypropylène (PP) et soumis à l'une des conditions atmosphériques suivantes : faible oxygène (4 % O₂ + 5 % CO₂), oxygène pur (100 % O₂) et air ambiant (contrôle). Nous avons évalué les effets d'atmosphères modifiées sur la qualité et l'évolution microbienne d'ananas fraîchement découpé. **Résultats et discussion.** Les traitements d'atmosphère modifiée ont tous les deux retardé la baisse de fermeté, des teneurs en composés solubles (SSC), en sucres réducteurs et en acide ascorbique. Les tranches d'ananas emballées sous O₂ pur ont produit des quantités plus faibles de sucre et d'acide ascorbique que les tranches d'ananas sous faible O₂, mais l'ananas fraîchement coupé a montré davantage de signes de brunissement sous O₂ pur. En outre, les traitements d'atmosphère modifiée ont fortement retardé la croissance des microorganismes. Les comptages de bactéries aérobies, de levures et de moisissures étaient plus nombreux dans les tranches d'ananas emballées sous O₂ pur qu'en faible O₂ sur une longue période de conservation. **Conclusion.** Le conditionnement sous atmosphère contrôlée utilisant une faible concentration d'oxygène (4 % O₂ + 5 % de CO₂) s'est révélée davantage en mesure de maintenir la qualité de l'ananas frais découpé que l'emballage sous O₂ pur.

Mots clés : ananas / *Ananas comosus* / produit frais découpé / conditionnement sous atmosphère contrôlée / qualité des aliments.

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1 Introduction

Fresh-cut pineapple (*Ananas comosus* (L.) Merr.) continues to increase in popularity because it is considered more convenient than the whole fruit [1]. However, current commercial fresh-cut pineapple products have a shelf-life of only 5–7 days at 1–7 °C, limited largely by the development of off-flavors and off-odors from physiological processes and microbial spoilage [2, 3]. At the same time, mechanical damage that occurs during fresh-cut processing also stimulates the generation of reactive oxygen species (ROS) including superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH\cdot$) [4]. Immediately following wounding, a rapid increase in oxygen uptake is followed by an initial burst of ROS production [5]. Excess ROS can cause lipid peroxidation, membrane damage, and senescence of the fruit tissue [6].

Modified atmosphere packaging (MAP) is a preservation technique currently used by the fresh-cut produce industry [7]. MAP typically consists of a combination of low levels of O_2 (2–6%) and an elevated level of CO_2 (7–15%). This mixture extends the shelf-life of fresh-cut pineapple by inhibiting fast-growing aerobes and slowing the respiration of living tissues [8, 9]. Low levels of O_2 and high levels of CO_2 reduce tissue respiration rate, with the benefit of delaying senescence and extending the storage life of the fresh produce [10]. In our previous research, we found that 4% O_2 + 5% CO_2 yielded better preservation effects for fresh-cut pineapple than ambient air. Product quality remained high after 9 days of storage at 10 °C [11]. However, it has been recognized that under certain conditions the growth of anaerobic psychrotrophic pathogens on fresh-cut produce might occur or even be stimulated by low-oxygen MAP [12]. In the last few years, high O_2 MAP has been suggested as an alternative to low O_2 atmospheres to inhibit the growth of naturally occurring spoilage microorganisms, prevent undesirable anoxic reparative processes, and maintain the high quality of fresh-cut produce [12–15]. Modified atmosphere with 50% O_2 + 50% CO_2 inhibited the growth and volatile metabolite production of *Candida argentea* and *C. sake* on pineapple agar. It also retarded the growth of aerobes and yeasts on pineapple cubes during storage [16]. A MAP of 80–100% O_2 also inhibited the *in vivo* growth of *Botrytis cinerea* on strawberries [17]. Application of pure oxygen significantly prevented fruit pericarp browning and delayed the increase in membrane permeability of litchi fruit during storage [18]. Nevertheless, sensitivity to O_2 toxicity varies between fruit species. Based on differences in browning times, we infer that increased O_2 concentrations around and within fruits may result in higher levels of ROS, such as superoxides, hydrogen peroxides and hydroxyl radicals, that can damage plant tissues and cause premature senescence [19].

However, as far as we know, there are no published data on pure O_2 modified atmospheres used for fresh-cut pineapple packaging. There are also no comparative studies of the differences between pure O_2 and conventional low O_2 concentrations on fresh-cut pineapple. The objective of this study was to evaluate the effect of a low oxygen MAP (4% O_2 + 5% CO_2) and a pure oxygen MAP on the quality of fresh-cut pineapple during storage.

2 Materials and methods

2.1 Plant material, treatments and storage

Fresh pineapples (*Ananas comosus* (L.) Merr. cv ‘Comte de Paris’) were obtained from a local wholesale market and stored at 10 ± 1 °C for 12 h before processing. The fruit was sorted to eliminate damaged or defective units and the selected pineapples were cleaned and the crowns removed. The pineapples in this experiment were at the 2–3 maturity stage according to the Dole pineapple color chart (between 25 and 50% shell color change).

The pineapples were peeled, halved, cored, and sliced transversely ($3.0 \times 2.0 \times 1.5$ cm) using a sharp, stainless steel knife. The slices were then disinfected for 5 min in a 100 mg L^{-1} chlorine solution and dried. The knife and cutting board were washed with 100 mg L^{-1} chlorine solution for 5 min prior to use. They were divided into three subgroups, and individually sealed by a tray sealer ($20.5 \times 13.0 \times 3.0$ cm) using a polyethylene (PE)/ polypropylene (PP) composite film (0.045 mm thickness). The following modified atmosphere treatments were applied:

- group 1: low oxygen (4% O_2 + 5% CO_2),
- group 2: pure oxygen (100% O_2),
- group 3: control at ambient air (21% O_2 + 0.03% CO_2),

all balanced with N_2 and stored at 10 ± 0.3 °C with 80% relative humidity. Each group consisted of 30 trays of about 150 g pineapple slices. The atmosphere was supplied by a modified atmosphere packaging machine (MAP-H360, Suzhou Kaikang Machinery Equipment Co., Ltd., Suzhou, China).

2.2 Gas analysis

Gas composition inside packages was determined with an O_2/CO_2 Dual Head Space Analyzer (Model PAC CHECK 325, Mocon, Minneapolis, MN, USA).

2.3 Quality evaluation

Fruit samples were stored and analyzed at time points just before packaging (1 day) and after storage for 3, 5, 7, 9, and 11 days. At each time point, three trays per treatment were randomly chosen and analyzed.

Firmness of the slices was measured with a GY-1 Firmness Tester (Mudanjiang Mechanical Institute, Mudanjiang, China). Firmness was reported as the penetration force required to depress a 3.5 mm cylinder probe 10 mm into the sample. Color was measured with a CR-400 chromometer (Konica Minolta, Japan) and expressed as L^* , a^* , and b^* , indicating luminance, chromaticity on a green (–) to red (+) axis, and chromaticity on a blue (–) to yellow (+) axis, respectively.

The content of reducing sugars was measured according to the methods of Han [20]. A 2 g sample of tissue was homogenized with distilled water and transferred to a 100 mL volumetric flask and brought up to volume with distilled water. The homogenate was extracted at room temperature for 20 min

and then filtered. Ten mL filtrate was mixed with 2.5 mL of 0.1 mol L⁻¹ iodine solution and 4.0 mL of 0.1 mol L⁻¹ sodium hydroxide (NaOH) in a conical flask. The conical flask was briefly shaken and placed in the dark for 15 min. Then 1 mL of 0.5 mol L⁻¹ HCl was added and the solution was titrated with 0.1 mol L⁻¹ sodium thiosulphate. When the solution became pale yellow, 5 drops of starch indicator were added, and the solution continued to be titrated until it was colorless.

Ascorbic acid content was measured by using the 2,6-dichlorophenol-indophenol dye titration method [20]. Pineapple samples (1 g) were homogenized with 50 mL of 2% (v/v) oxalic acid. The mixture was then filtered for 15 min. Ten mL filtrate were titrated against 0.01% (w/v) 2,6-dichlorophenolindophenol dye that was standardized using an ascorbic acid standard. The ascorbic acid content was expressed as mg 100 g⁻¹ fresh weight (FW).

Soluble solids content (SSC) was determined by extracting one drop of juice from the pineapple sample and measured with a digital refractometer (Atago Co. Ltd., WYT-1, Tokyo, Japan).

2.4 Microbial analysis

Aerobic bacterial counts, mold and yeast numbers were determined with a Film plate TM Aerobic count plate, and a Film plate TM mold & yeast count plate (Guangzhou Oasis Biochemical Technology Co., Ltd., Guangzhou, China). A 1 mm thick layer of pineapple slice was removed with a sterilized stainless steel knife and a 1 g sample was ground with a sterilized mortar in an ultraclean laboratory. A 1:10 solution was prepared by mixing this sample with 9 mL sterilized saline. Then a 1:100 solution was prepared by pipetting 1 mL of the 1:10 solution into 9 mL of sterilized saline, and so on, to prepare a dilution series of the solution. The TM count film plate was placed on the sterile experiment table, the film was uncovered on the surface, and 1 mL solution was added to the plate. The film was covered gently and allowed to stand for 5 min until the solution dried. Two plates were inoculated for each dilution ratio. The plates were stacked (fewer than 12 plates per pile) and stored in their original plastic bags, with the film side upward. The bacterial plates were cultured for 24 h at 36 ± 1 °C and the mold and yeast plates were cultured for 48–72 h at 29 ± 1 °C.

Colonies appeared red on the aerobic count plate and we counted the colony number by choosing a plate containing 10 to approximately 100 colonies. Mold and yeast appeared blue after growing on the plate. Mold colonies were radial and relatively large compared with round yeast colonies. We counted the colony number by choosing a plate that contains 10–100 colonies. The colony numbers were counted as the film TM aerobic count plate manufacturer's instructions and expressed as colony forming units per gram fresh weight (cfu g⁻¹ FW). Data from all colonization experiments were transformed with the log cfu g⁻¹ FW transformation.

2.5 Statistical analysis

All experiments were conducted in triplicate. All data points represent the mean ± standard deviation (SD) of all

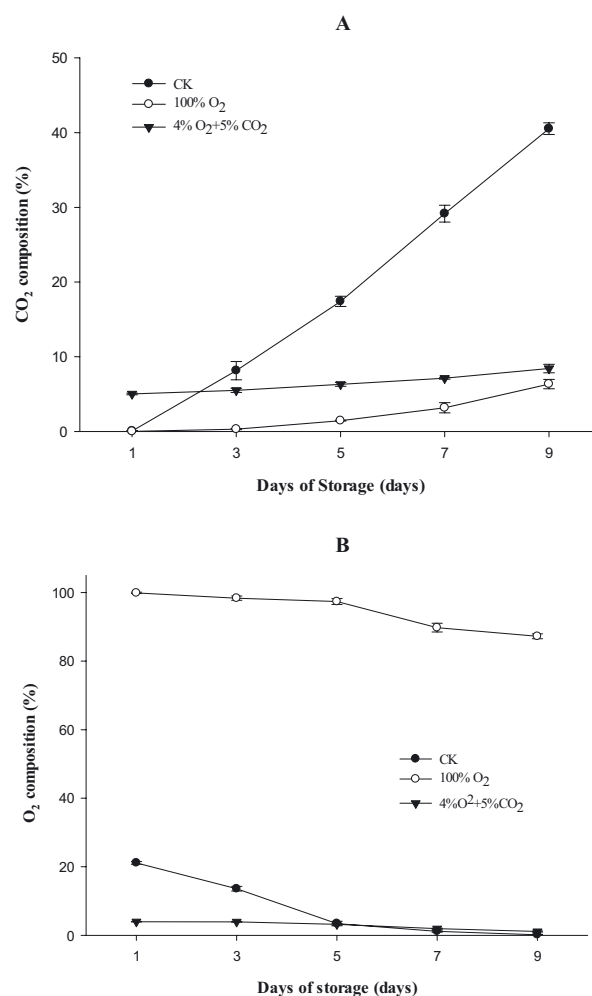


Figure 1. Evolution of CO₂ (A) and O₂ (B) concentrations measured in different MAP packaging of fresh-cut pineapple stored for 9 days at 10 °C (CK: control at ambient air). Data presented are the means of three replicates. Vertical bars represent the SD.

replicates. One-way repeated measures and multivariate analysis of variance (ANOVA) of the general linear model for least significant differences (LSD) $P < 0.05$ were performed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA).

3 Results and Discussion

3.1 Packaging atmosphere

The O₂ content decreased and CO₂ content increased in fresh-cut pineapple kept in modified atmosphere packaging at 10 °C (figure 1). The CO₂ concentration increased by 40.93% in air packs, whereas the CO₂ concentration increased only 5–8% in the packs of 4% O₂ + 5% CO₂. This increase in CO₂ concentration was slightly lower than in the packs of 100% O₂. However, O₂ concentration declined from 21% to 0.18% in air packs during the 9 day storage. The O₂ concentration decreased only from 5% to 1.71% in the packs of 4% O₂ + 5% CO₂. O₂ concentration in the packs of 100% O₂

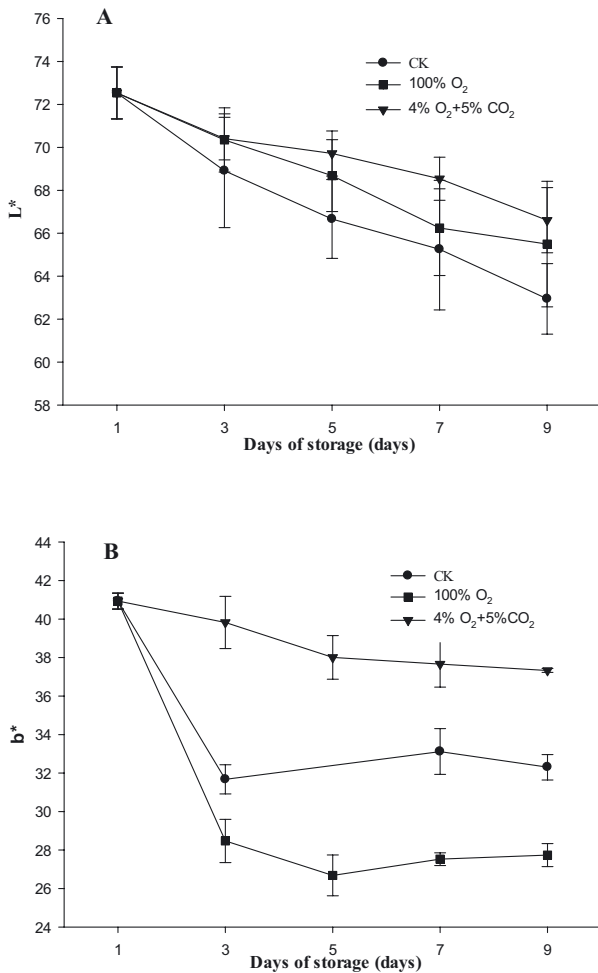


Figure 2. Evolution of (A) luminance (L^*) and (B) chromaticity on a blue (–) to yellow (+) axis (b^*) in different MAP packaging treatments of fresh-cut pineapple after 9 days of 10 °C storage (CK: control at ambient air). Data presented are the means of three replicates. Vertical bars represent the SD.

remained as high as 87.24% after 9 days. According to Soliva-Fortuny *et al.* [21], a decrease in O₂ levels below a fermentative threshold limit of 2% could induce anaerobic respiration, which would result in the production of off-flavors and odors. We found that the control sample began to exhibit an alcoholic flavor within 5 days. At the same time, the oxygen concentration in these packages had dropped to 1.43%. The change in gas composition in the MAPs is dependent on the plant tissue respiration [22]. Thus, our results indicate that MAPs with pure O₂ and MAPs with low concentration of O₂ (4%) and high concentration of CO₂ (5%) were successful at inhibiting respiration in fresh-cut pineapple.

3.2 Effect of MAP on the color of fresh-cut pineapple

The color of fresh-cut pineapple was progressively browner during storage than that of just-cut pineapple for all the samples, as confirmed by luminosity decreases (*figure 2*).

This is a consequence of browning during storage [23–25], a phenomenon caused by phenolic oxidation, which is catalyzed by polyphenoloxidase enzymes to form colored melanins [26].

Compared with the control sample, the 100% O₂ samples and low O₂-high CO₂ significantly reduced decreases in luminance value during storage ($P < 0.05$). The luminance value reduction in pineapple slices in the low O₂ MAP was the slowest (*figure 2A*). The parameter of chromaticity on a blue (–) to yellow (+) axis decreased sharply after the first day of storage, but it showed a slight increase from 3 days to 5 days (in the ambient air and pure O₂ groups) or a slow decline in the low O₂ group (4% O₂ + 5% CO₂) (*figure 2B*). As reported in previous studies, a slow decline in chromaticity could be due to the surface dehydration of the product that preludes tissue senescence [23]. During the storage process, the chromaticity on a blue (–) to yellow (+) axis value of pineapple slices in low oxygen MAP experienced only a slight drop. However, the chromaticity of pineapple packaged in pure O₂ declined the most.

On the one hand, our results show that a MAP with low concentrations of O₂ (4%) combined with high concentrations of CO₂ (5%) significantly reduced browning of fresh-cut pineapple. Similar results have also been reported by Marrero and Kader [27], who also found that reduced O₂, with or without increased CO₂, improved the retention of yellow color of fresh-cut pineapple. On the other hand, we also found that a MAP with pure O₂ actually increased browning. High oxygen concentrations was also shown to be detrimental to the fruit quality of fresh-cut strawberries [28]. However, mushroom flesh and surfaces exposed to 80% O₂ were prevented from browning [29]. A similar result was also obtained by Limbo and Piergiovanni with potatoes [30]. Zheng *et al.* [31] and Duan *et al.* [18] also found that a high oxygen treatment reduced browning in loquat and litchi fruit respectively. Thus, the effects of a high oxygen MAP treatment on browning depends on the commodity, maturity, O₂ concentration, and temperature.

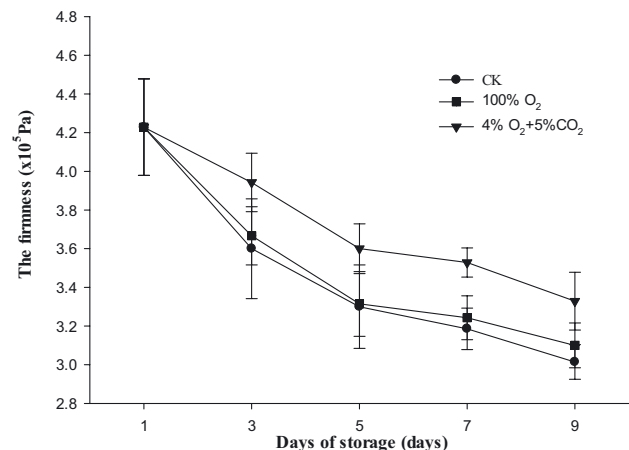


Figure 3. Change of firmness in different MAP packaging treatments of fresh-cut pineapple after 9 days of 10 °C storage (CK: control at ambient air). Data presented are the means of three replicates. Vertical bars represent the SD.

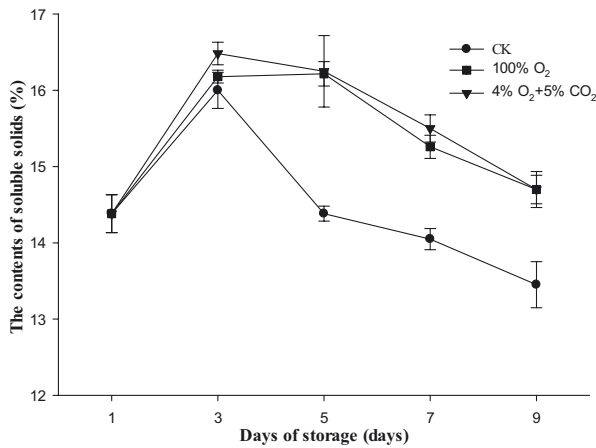


Figure 4. Change of soluble solid contents (SSC) in different MAP packaging treatments of fresh-cut pineapple after 9 days of 10 °C storage (CK: control at ambient air). Data presented are the means of three replicates. Vertical bars represent the SD.

3.3 Effect of MAP on firmness of fresh-cut pineapple

The firmness of the fresh-cut pineapple all showed a decreasing trend, but the slices packaged in ambient air and pure O₂ decreased faster compared with firmness in the samples packaged in 4% O₂ + 5% CO₂ (figure 3). Further statistical analysis showed no significant differences ($P < 0.05$) between the pure O₂ and control ambient air packaged pineapple. Fernández-León *et al.* [32] also found 10% O₂ and 5% CO₂ delayed firmness decrease of broccoli crowns.

3.4 Effect of MAP on nutrition quality of fresh-cut pineapple

All pineapple MAP packaging treatments exhibited a downward trend after an initial increase in SSC (figure 4). The SSC in the slices packaged in pure O₂ and 4% O₂ + 5% CO₂ was significantly lower than the control group. There was no significant difference ($P < 0.05$) between the pure O₂ and 4% O₂ + 5% CO₂ groups. The rise of SSC in the early stage of storage was surprising but the following decline due to respiratory consumption occurred as expected. Santos *et al.* [33] also found that fresh-cut “Pérola” pineapple retained higher values of SSC under a 5% O₂ + 5% CO₂ modified atmosphere. In contrast, Montero-Calderón *et al.* [34] found that SSC values showed little change in fresh-cut pineapple (*Ananas comosus*) during storage and no significant differences were found over time or among packaging conditions. Similar results have also been reported in fresh-cut pineapple (‘Gold’ cultivar) stored under modified atmosphere conditions of 2% O₂ and 10% CO₂ [35].

Sugar is one of the main nutrients in the pineapple fruit. As illustrated in figure 5, all of the pineapple groups showed a downward trend in sugar content. However, pineapple packaged in modified atmospheres had a slower decline in sugar than the pineapple slices packaged in ambient air. The sugar content in pineapple packaged in 4% O₂ + 5% CO₂ declined

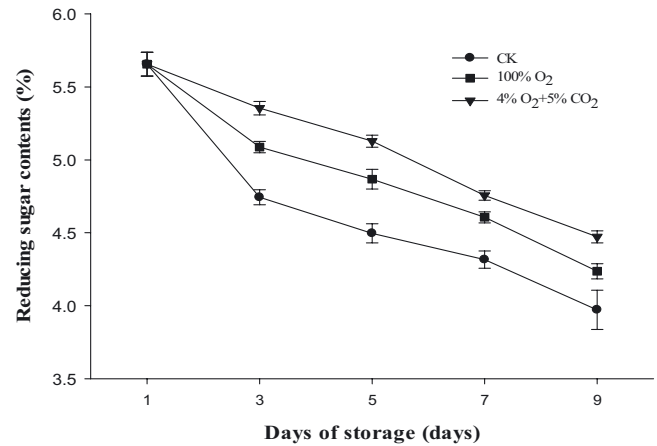


Figure 5. Change of reducing sugar content in different MAP packaging treatments of fresh-cut pineapple after 9 days of 10 °C storage (CK: control at ambient air). Data presented are the means of three replicates. Vertical bars represent the SD.

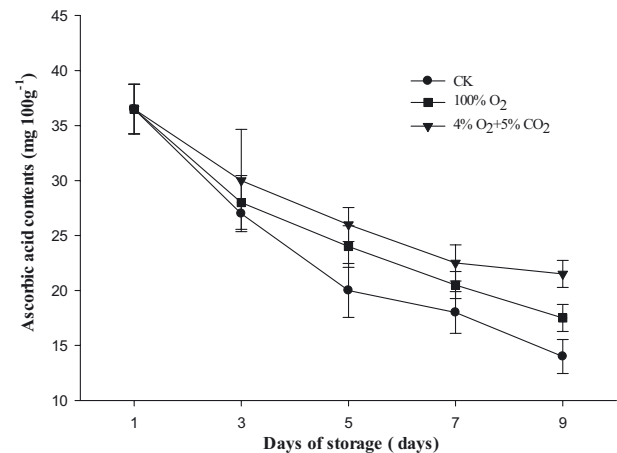


Figure 6. Change of ascorbic acid in different MAP packaging treatments of fresh-cut pineapple after 9 days of 10 °C storage (CK: control at ambient air). Data presented are the means of three replicates. Vertical bars represent the SD.

most slowly. Between the first measurement and the end of the storage time, the sugar content of the ambient air control group, the pure oxygen MAP group and the low O₂ group fell to 27.2%, 24.6% and 17.5%, respectively.

The content of ascorbic acid (AA) of fresh-cut pineapple decreased in all samples during the course of the study (figure 6). However, pure O₂ and low O₂ modified atmospheres both slowed the decline of AA content. The low O₂ MAP was most effective. A similar result was found in broccoli [36]. The magnitude of these changes may be directly related to the O₂ concentration inside the packages, as reported by Soliva-Fortuny *et al.* [37]. Hence, higher concentrations of O₂ in the bag headspace results in larger decreases in AA. The reason for this relationship between AA content and O₂ level in MAP needs further research.

3.5 Effect of MAP on microbial stability in fresh-cut pineapple

Aerobic bacterial counts can reflect the freshness of the produce and the hygienic conditions of the fresh-cut production facility. As illustrated in *figure 7A*, the aerobic bacterial count in pineapple slices packaged in pure O₂ and those packaged in low concentration O₂ only reached 3.9 and 4.5 log cfu g⁻¹ respectively, while slices packaged in ambient air reached 5.9 log cfu g⁻¹ by the end of the storage time. This result suggests that a conventional MAP (4% O₂ + 5% CO₂) and a super atmospheric O₂ MAP both contribute to inhibiting the growth of microorganisms. Similar results have also been reported by Oms-Oliu *et al.* [38], who found that both (2.5 kPa O₂ + 7 kPa CO₂) and (70 kPa O₂) atmospheres significantly reduced the growth of microorganisms in fresh-cut melon. In addition, low O₂ and moderate CO₂ atmospheres can slightly reduce the microbial growth of fresh-cut cantaloupe and honeydew melons [39]. Fifty percent O₂ combined with 50% CO₂ also decreased the growth of aerobes on pineapple cubes during storage [16].

The growth of yeasts and molds was also markedly decreased on pineapple slices packaged in pure O₂ and those packaged in low concentration O₂ modified atmospheres (*figure 7B, 7C*). By the end of the storage time (day 9), the slices packaged in low concentration O₂ had the lowest mold and yeast growth, followed by those packaged in pure O₂. Slices packaged in ambient air experienced the highest mold and yeast growth. In agreement with our results, Oms-Oliu *et al.* [38] also found that yeast and mould growth on fresh-cut melon stored under (2.5 kPa O₂ + 7 kPa CO₂) or (70 kPa O₂) atmospheres were inhibited until the third week of storage.

On the other hand, high oxygen concentration has been reported to stimulate the growth of microorganisms on fresh-cut fruit and vegetables [40]. Enterobacteria on sliced carrots were inhibited under 50% O₂ + 30% CO₂, but stimulated under 80 or 90% O₂. Poubol and Izumi [41] also reported that a high O₂ of 60 kPa stimulated the growth of aerobic bacteria on “Carabao” mango cubes and yeasts on ‘Nam Dokmai’ mango cubes at 13 °C. In their opinion, stimulated growth of bacteria under high O₂ may be influenced by the pH of the fruit surface [41].

Other research has shown that high oxygen has no effect on microbial growth. Growth of yeasts and anaerobic mesophilic bacteria was not affected by varying oxygen during the packaging of mushroom slices [42]. The effects of high oxygen on microorganism growth may be related to the microbial species present and the food substrate.

4 Conclusions

The major factors that shorten the shelf life of fresh-cut pineapple are those that increase fruit deterioration and make the fruit vulnerable to infection by microorganisms. MAP using low oxygen (4% O₂ + 5% CO₂) and pure oxygen both delayed the decrease of firmness, SSC, reducing sugars, and AA in fresh-cut pineapple. However, MAP using pure O₂ exhibited a faster decline of sugar and AA. Furthermore, MAP

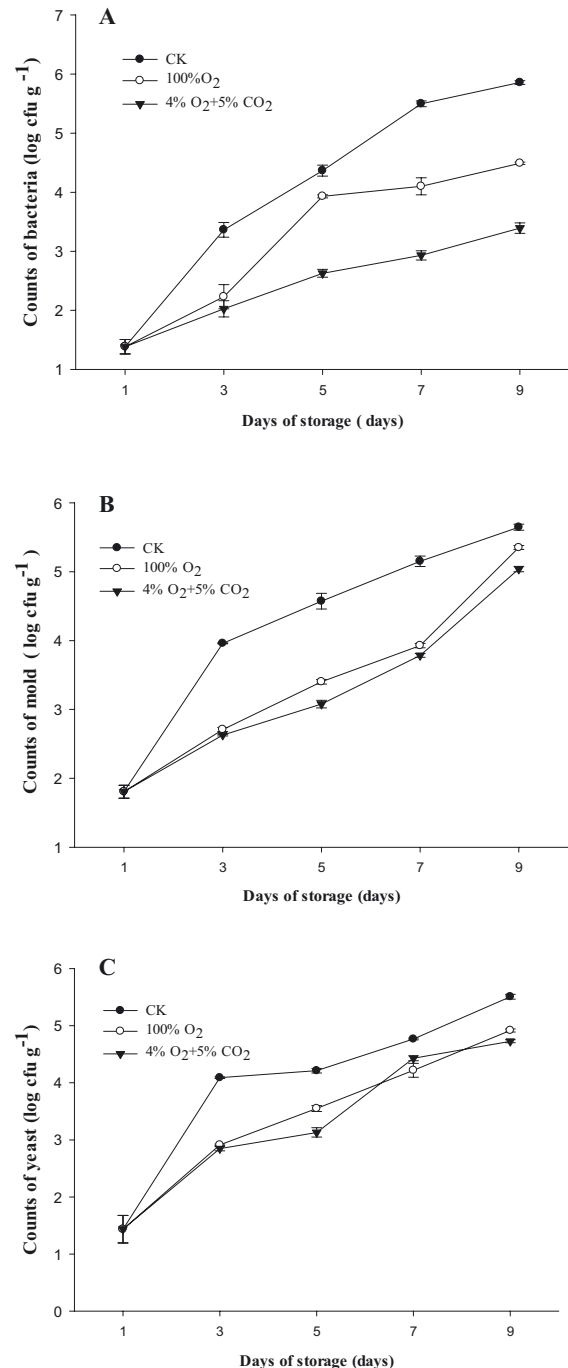


Figure 7. Changes of (A) aerobic bacterial (B) mold, and (C) yeast counts in different MAP packaging treatments of fresh-cut pineapple after 9 days of 10 °C storage (CK: control at ambient air). Data presented are the means of three replicates. Vertical bars represent the SD.

using pure O₂ increased browning of the fresh-cut pineapple. Both MAP treatments strongly decreased the aerobic bacterial count and decreased the growth of yeasts and mold. However, pineapple slices packaged in pure O₂ had higher aerobic bacterial counts and more yeasts and mold than those packaged in low O₂ concentrations (4% O₂ + 5% CO₂). Overall, the use of MAPs with conventional low O₂ concentrations is

more effective for maintaining the quality of fresh-cut pineapple than MAPs using pure O₂.

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