

# Influence of Modified Atmosphere Packaging on Volatile Compounds and Physicochemical and Antioxidant Attributes of Fresh-Cut Pineapple (*Ananas comosus*)

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The effects of modified atmosphere packaging on volatile compound content and physicochemical and antioxidant attributes of Gold cultivar fresh-cut pineapples were assessed throughout storage at 5 °C. Fresh-cut pineapple pieces were packed under LO (low oxygen, 12% O<sub>2</sub>, 1% CO<sub>2</sub>), AIR (20.9% O<sub>2</sub>) and HO (high oxygen, 38% O<sub>2</sub>) headspace atmospheres. Methyl butanoate, methyl 2-methylbutanoate, and methyl hexanoate were the most abundant volatiles regardless of the packaging atmosphere and days of storage; whereas most odor active volatiles were methyl and ethyl 2-methylbutanoate, 2,5-dimethyl-4-methoxy-3(2*H*)-furanone and ethyl hexanoate. Physicochemical attributes of pineapple did not significantly vary, whereas vitamin C content and total antioxidant capacity were lower for fresh-cut pineapple in HO (488 ± 38 mg/100 mg<sub>fw</sub> and 54.4 ± 5.7%, respectively) than for LO and AIR packages. Storage life of fresh-cut pineapple was limited to 14 days by volatile compounds losses and fermentation processes.

KEYWORDS: Pineapple; aroma profile; volatile compounds; modified atmosphere packaging; antioxidant properties; *Ananas comosus* 

## INTRODUCTION

Pineapple is one of the most popular tropical fruits. Its flesh is nutritious, juicy, aromatic and very tasty. However, it is a large fruit which requires labor and space for processing and storage. This inconvenience can be avoided by fresh-cut pineapple products, ready-to-eat, with the freshness of the intact fruit. Nonetheless, the quality of fresh-cut fruits rapidly deteriorates after processing.

Modified atmospheres have been used as alternative treatments to increase the shelf life of fresh-cut products. Reduced oxygen and increased carbon dioxide levels in package headspace can help to slow down respiration reactions as well as changes in color, texture and other quality attributes, but they have been shown to cause changes in flavor volatile content in whole citrus, apples and mangoes and their fresh-cut derivatives (1). Although some data are available on the effect of the use of modified atmospheres on pineapple (2-4), no information has been published on the effects of storage atmosphere on the volatile compounds emitted by pineapple.

Most efforts to preserve the quality of fresh-cut products have been done on appearance and safety attributes, but flavor has become a key factor in consumer preferences and buying decisions. Moreover, Kader (5) suggested that flavor attributes are usually lost before other deterioration symptoms appear. Flavor has two components: aroma and taste. Aroma is the result of the combined effect of the presence of various volatiles in the fruit, and taste, the result of content of nonvolatile compounds, thus it is necessary to understand how they change throughout storage.

Pineapple aroma has been studied for many years; most works have been focused on compound identification, which has led to over 400 compounds identified in fresh and processed products (6-9). There is some information on changes with maturity stage and stress treatments for some cultivars (10-14); however, cultivars are not always reported, extraction procedures and analyses vary and quantification of volatiles is reported using different relative units, making it difficult to compare. Odor activity values were reported by Tokitomo et al. (9), who found 4-hydroxy-2,5 dimethyl-3(2H)-furanone, ethyl 2-methylpropanoate, ethyl 2-methylbutanoate as the main contributors to pineapple aroma for the super sweet cultivar (F-2000), with odor activity values above 1000. In preliminary tests, we found methyl butanoate, methyl 2-methylbutanoate, and methyl hexanoate were the most abundant components of Gold cultivar pineapple flesh; whereas the largest contributors to pineapple aroma were methyl 2-methylbutanoate, mesifurane (2,5 dimethyl-4methyoxy-3(2H)-furanone) and ethyl 2-methylbutanoate. Nonetheless, there is limited information on volatile composition variations of fresh-cut pineapple throughout storage.

The objective of this study was to evaluate the effect of modified atmosphere packaging on the volatile compounds content and odor activity, physicochemical and antioxidant attributes of Gold cultivar fresh-cut pineapple throughout storage at 5  $^{\circ}$ C.

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#### MATERIALS AND METHODS

**Materials.** Gold cultivar pineapples (*Ananas comosus* L. Merrill) imported from Costa Rica were bought at a local supermarket in Lleida, Spain, and stored at  $11 \pm 1$  °C overnight prior to processing. Fruits were free from mechanical injuries, insects, pathogens or other defects. Shells had several to most of their eyes partially filled with yellow color, all of them surrounded by green.

Polypropylene trays (500 cm<sup>3</sup>, MCP Performance Plastic Ltd., Kibbutz Hamaapil, Israel) were sealed with a 64  $\mu$ m thickness polypropylene film (Tecnopack SRL, Mortara, Italy) with a permeability to O<sub>2</sub> and CO<sub>2</sub> of 110 and 550 cm<sup>3</sup>/m<sup>2</sup> /bar/day at 23 °C and 0% relative humidity, respectively.

Chemicals. Authentic volatile compounds were used as internal and external standards for fresh-cut pineapple aroma analysis. They were chosen from previous studies with pineapple products. The list of chemicals is given ahead, followed by the odor threshold concentration in water  $(\mu g/kg)$  of each volatile compound, when available. Methyl salicylate (internal standard), and the following external standards: (1) methyl 2-methylpropanoate, 6.3  $\mu$ g/kg (9); (2) ethyl propanoate; (3) methyl butanoate,  $72 \,\mu g/kg(6)$ ; (4) ethyl 2-methylpropanoate; (5) methyl 3-methylbutanoate; (6) methyl 2-methylbutanoate, 0.1  $\mu$ g/kg (6); (7) hexanal; (8) butyl acetate; (9) ethyl 2-methylbutanoate,  $0.006 \mu g/kg$  (6); (10) 3-methylbutyl acetate, 2 µg/kg (15); (11) 2-heptanone; (12) methyl 5-hexenoate; (13) methyl hexanoate,  $77 \,\mu g/kg (15)$ ; (14) ethyl hexanoate, 1  $\mu$ g/kg (15); (15) hexyl acetate; (16) methyl 3-(methylthio)propanoate, 180  $\mu g/kg$  (15); (17) limonene, 10  $\mu g/kg$  (15); (18) (Z)-beta-ocimene; (19) 2,5-dimethyl-4-hydroxy-3(2H)-furanone; (20) 2,5-dimethyl-4-methoxy-3(2H)-furanone, 0.03 µg/kg (15); (21) ethyl heptanoate, 2.2 µg/kg (15); (22) ethyl 3-(methylthio)propanoate; (23) linalool; (24) nonanal, 1  $\mu$ g/ kg (15); (25) methyl octanoate, 200 µg/kg (15); (26) 4-ethylphenol; (27) methyl (E)-2-octenoate; (28) ethyl octanoate; (29) geraniol; (30) 4-ethyl-2-methoxyphenol; (31) ethyl decanoate; (32) alpha copaene. Reagents were purchased from Sigma-Aldrich Química SA, Madrid, Spain.

**Fresh-Cut Processing.** Working area, cutting boards, knives, containers and other utensils and surfaces in contact with the fruit during processing were washed and sanitized with  $200 \,\mu$ L/L sodium hypochlorite solution at pH 7 to have a maximum sanitizing effect before processing. Pineapple crown leaves were removed, and the fruit was washed twice in two  $200 \,\mu$ L/L sodium hypochlorite solutions for 5 min each, letting excess water drain for 3–5 min after each dip. Fruits were peeled and cut into 1.2 cm thick slices using an electric slicing machine (Food Slicer-6128: Toastmaster Corp, Elgin, IL). Slices were cored and cut into wedges (6–7 g, each) with sharp knives.

Fruit pieces from the bottom, middle and top sections of the fruit were carefully mixed before packaging to minimize the effect of flesh quality differences along the fruit. Fresh-cut pineapple pieces were immersed in 1% citric acid and 1% ascorbic acid solution for 2 min as antibrowning agents and to keep the surface pH low enough to reduce microbial growth. Excess water was drained for 2 min, and 100 g pineapple pieces were packaged under the following initial conditions: (a) LO (low oxygen; 12%  $O_2$  and 1% CO<sub>2</sub>), (b) AIR (20.9%  $O_2$ ), and (c) HO (high oxygen; 38%  $O_2$ ).

Trays were sealed using a vacuum sealer (ILPRA Foodpack Basic V/G, Ilpra, Vigenovo, Italy) and kept at 5 °C for up to 25 days. For each tray, a fruit weight to volume ratio of 2:10 g/mL was used. Two trays (100 g of fresh-cut pineapple) from each packaging condition were randomly selected at each sampling date for headspace gas composition analysis, volatiles content, SSC (soluble solids content), TA (titratable acidity), and pH, flesh color, juice leakage, vitamin C, total phenolic content and antioxidant capacity.

**Quality Evaluation.** Packages' internal atmosphere, headspace volatile compounds, nonvolatile content and microbiological stability were evaluated on fresh-cut pineapple along storage.

**Package Headspace Analysis.** The headspace oxygen, carbon dioxide, ethylene, ethanol and acetaldehyde composition of each single tray was analyzed using a gas chromatograph equipped with a thermal conductivity detector (Micro-GP CP 2002 gas analyzer, Chrompack International, Middelburg, The Netherlands) as described by Rojas-Graü et al. (13). A 1.7 mL aliquot was withdrawn through an adhesive septum stuck to the film cover, with a sampling needle directly connected to the injection module. The determination of the O<sub>2</sub> concentration was carried out by injecting a sample of 0.25  $\mu$ L to the a CP-Molsieve 5 Å packed

column (4 m × 0.32 mm, d.f. = 10 mm) at 60 °C and 100 kPa, whereas a portion of 0.33  $\mu$ L was injected into a pora-PLOT Q column (10 m × 0.32 mm, d.f. = 10 mm) held at 75 °C and 200 kPa for CO<sub>2</sub>, ethylene, acetaldehyde and ethanol determinations.

**Volatile Components Analysis.** Volatile components of fresh-cut pineapple were extracted by headspace solid-phase microextraction (SPME) using a polydimethylsiloxane (PDMS) fiber with a 100  $\mu$ m thickness coating from Supelco Co. (Bellefonte, PA), followed by gas chromatography/mass spectrometry similar to that described by Lamikanra and Richard (*14*). Two trays with 100 g of fresh-cut pineapple packaged in LO, AIR and HO atmospheres were evaluated after 0, 7, 14, and 21 days of storage at 5 °C.

Fruit pieces from each tray were homogenized using an Ultra Turrax T25; two 4 g samples of each homogenate were placed into 20 mL clear glass vials. Methyl salicylate (CAS number 119-36-8) in water solution was added as internal standard (500  $\mu$ g/kg). Vials were sealed and stirred for 15 min at 30 °C to achieve partition equilibrium of the analytes between the sample and the headspace; then the SPME fiber was inserted through a PTFE-faced butyl septum of cap into the headspace of the vial and held for 15 min (sampling time) while stirring was continued.

Adsorbed substances were desorbed by inserting the PDMS fiber into the gas chromatograph—mass spectrometer (GC–MS) injection port at 250 °C. The desorbed compounds were separated using an Agilent 6890 N gas GC coupled to a 5973 mass selective detector (Agilent Technologies España, S.L., Las Rozas, Spain) equipped with a Supelco Equity 5 capillary column of 30 m × 0.25 mm i.d. coated with 0.25  $\mu$ m thick poly (5% diphenyl/95% dimethylsiloxane) phase (Supelco, Bellefonte, PA). Extraction temperature (30 °C) was chosen with the aim to reproduce naturally occurring aroma profile of fresh pineapple.

The GC was operated in a splitless mode using helium as the carrier gas at a constant rate of 1.5 mL/min. The oven temperature was programmed with an initial temperature of 40 °C, followed by a ramp up to 250 at 20 °C/min and held for 10 min at the final temperature. Mass spectra were obtained by electron ionization (EI) at 70 eV, and spectra range from 40 to 450 m/z.

The SPME fiber was preconditioned at 200 °C for 15 min before each use, and blank runs were done to check the absence of residual compounds on the fiber.

Identification of volatile compounds in pineapple was performed by comparison of mass spectra and retention times of target compounds with that of authentic reference substances. Thirty-two authentic reference compounds were used to identify and quantify volatile components in fresh-cut pineapple. Aqueous solutions with known concentration of reference volatiles were analyzed using headspace solid-phase micro-extraction with a 100  $\mu$ m PDMS coating fiber, followed by GC-MS analysis using identical conditions to those used for pineapple samples.

Quantification was done by the calculation of average relative response factors (RRF) for each volatile compound, using the chromatographic data of prepared water solutions with respect to methyl salicylate, used as internal standard (RRF = peak area<sub>analyte</sub> × concentration<sub>int.std</sub>/peak area<sub>int.std</sub>. × concentration<sub>analyte</sub>).

Aroma profile was defined by the volatiles detected in fresh-cut pineapple under extraction and analysis conditions. Volatiles concentrations throughout storage were determined for all packaging conditions. Volatiles odor contribution to pineapple aroma was assessed by odor activity values (OAVs), calculated as the ratio of actual volatile content to odor threshold concentration in water, given by the literature (9, 15, 16).

**Nonvolatile Components of Pineapple.** Titratable acidity, pH, and soluble solids content (%) were determined from duplicate 100 g samples of fresh-cut fruit, homogenized using an Ultra Turrax T25 (IKA WERKE, Germany) and filtered (Whatman paper No. 1). Soluble solids content was determined using an Atago RX-1000 refractometer (Atago Company Ltd., Japan), pH was directly measured using a pH-meter Crison 2001 (Crison Instruments S.A., Barcelona, Spain) and flesh acidity was assessed by titration with 0.1 N NaOH to a pH end-point of 8.1, and its results were expressed as grams of anhydrous citric acid per 100 g of fruit fresh weight. All measurements were carried out according to AOAC procedures. SSC/TA ratio was calculated for all packaging conditions and evaluation date.

Color was measured directly with a Minolta CR-400 chroma meter (Konica Minolta Sensing, INC. Osaka, Japan), using the CIE color space  $L^*a^*b^*$ . The equipment was set up for illuminant D<sub>65</sub> and 10° observer

angle and calibrated using a standard white reflector plate. Sixteen color readings were registered for each section of the fruit. Results were reported as  $L^*$ ,  $a^*$ , and  $b^*$ .

Juice leakage was determined as described by Montero-Calderón et al. (2). Trays were tilted at a 20° angle for 5 min and accumulated drained juice collected with a 5 mL syringe. Results were reported as liquid volume recovered per 100 g of fresh-cut fruit in the package.

Pineapple vitamin C content, total phenolic compounds content, and antioxidant capacity were measured on duplicated samples. Vitamin C extraction procedure was based on the method proposed by Odriozola-Serrano et al. (17). A portion of 25 g of fruit was added to 25 mL of a 4.5% metaphosphoric acid solution with 0.72% of DL-1,4-dithiothreitol (DTT) as reducing agent. The mixture was crushed, homogenized and centrifuged at 22100g for 15 min at 4 °C. The supernatant was vacuum-filtered through Whatman No. 1 filter paper. The samples were then passed through a Millipore  $0.45 \,\mu\text{m}$  membrane and injected into the HPLC system. Samples were introduced onto the column through a manual injector equipped with a sample loop ( $20 \,\mu$ L). Separation of ascorbic acid was performed using a reverse-phase C18 Spherisorb ODS2 (5 µm) stainless steel column (4.6 mm  $\times$  250 mm). The mobile phase was a 0.01% solution of sulfuric acid adjusted to pH 2.6. The flow rate was fixed at 1.0 mL/min. Detection was performed with a 486 absorbance detector (Waters, Milford, MA) set at 245 nm. Identification of ascorbic acid (Scharlau Chemie, SA, Barcelona, Spain) was carried out by HPLC comparing the retention time with those of the standards. Results were expressed as mg of vitamin C in 100 g of pineapple flesh.

Total phenolic content was determined by the colorimetric method described by Singleton et al. (18) using the Folin–Ciocalteu reagent. Fresh-cut pineapple samples were homogenized using an Ultra Turrax T25. The homogenate was centrifuged at 6000g for 15 min at 4 °C (Centrifuge Medigifer: Select, Barcelona, Spain) and filtered through a Whatman No. 1 filter paper. Then, 0.5 mL of the extract was mixed with 0.5 mL of Folin–Ciocalteu reagent, 10 mL of saturated Na<sub>2</sub>CO<sub>3</sub> solution and distilled water to complete 25 mL. Samples were allowed to stand for 1 h at room temperature before the absorbance at 725 nm was measured. Total phenolic content was determined by comparing the absorbance of duplicated samples with that of gallic acid standard solutions. Results were expressed as milligrams of gallic acid per 100 g of pineapple flesh.

The antioxidant capacity of pineapple flesh was determined using the method described by Odriozola-Serrano et al. (17), by measuring the free radical-scavenging effect on DPPH (1,1-diphenyl-2-picrylhydrazyl) radical. Duplicated samples were homogenized using an Ultra Turrax T25. The homogenate was centrifuged at 6000g for 15 min at 4 °C (centrifuge Medigifer: Select, Barcelona, Spain); 0.01 mL aliquots of the supernatant were mixed with 3.9 mL of methanolic DPPH solution (0.025 g/L) and 0.090 mL of distilled water. The homogenate was shaken vigorously and kept in the darkness for 30 min. Absorption at 515 nm was measured on a spectrophotometer (CECIL CE 201; Cecil Instruments Ltd. Cambridge, U.K.) against a methanol blank. Results were expressed as percentage decrease with respect to the initial value.

**Data Analysis.** Significance of results and statistical differences were analyzed using Statgraphics Plus version 5.1 (Statistical Graphics Co., Rockville, MD). Analysis of variance (ANOVA) was performed to compare quality attributes of fresh-cut pineapple throughout 5 °C storage, using the Duncan test to compare means at a 5% significance level.

### **RESULTS AND DISCUSSION**

**Package Headspace Gases.** Oxygen and carbon dioxide headspace concentration throughout storage at 5 °C are shown in **Figure 1**. Initial atmosphere concentration significantly affected the headspace atmosphere. During the first two weeks of storage, oxygen concentration decreased whereas carbon dioxide content increased, as a result of the metabolic activity of the fresh-cut fruit, together with some gases exchange through the package sealed film.

Fresh-cut fruits packed under LO atmospheres were the fastest to reach oxygen contents close to 2% (5 days), and they were followed by fruit pieces under AIR (7 days) and HO (15 days) atmospheres. In contrast, carbon dioxide content showed a steady increase in the headspace atmosphere until a plateau was



**Figure 1.** Headspace oxygen and carbon dioxide concentrations of Gold cultivar fresh-cut pineapple packaged under three initial atmospheres and stored at 5 °C. LO: 12% O<sub>2</sub>, 1% CO<sub>2</sub>. AIR: 20.9% O<sub>2</sub>. HO: 38% O<sub>2</sub>. Each point in the graph is the mean value of four measurements.



Figure 2. Ethanol content in packages' headspace of Gold cultivar freshcut pineapple throughout storage at 5 °C. LO: 12%  $O_2$ , 1%  $CO_2$ . AIR: 20.9%  $O_2$ . HO: 38%  $O_2$ . Each point in the graph is the mean value of four measurements.

reached during the second week of storage, which was later followed by an abrupt increase by the 19th day of storage, attributed to pineapple tissues' switch to anaerobic respiration. Changes occurred faster for LO and AIR packages, as compared with those with HO atmospheres.

Our findings showed that fresh-cut pineapple was able to tolerate low oxygen (2% or less) and high carbon dioxide concentrations (up to 25%) for several days, before switching to anaerobic respiration. Fruits packed under LO and AIR tolerated such conditions from the seventh to the 19th day of storage, without ethanol or acetaldehyde production increase. Thus, it is likely that the use of alternative packages with higher permeability characteristics could be used to reduce  $O_2$  depletion and  $CO_2$  accumulation inside the package headspace, and consequently retard fermentation reactions due to anaerobic behavior.

Ethanol and acetaldehyde headspace content are shown in **Figures 2** and **3**. Small accumulations of both gases were observed during the first weeks of storage. This was attributed to natural occurrence of both compounds in almost every fruit even under aerobic conditions (*19*), though larger accumulation of both gases also revealed some fermentation process.

Ethanol production in the trays was triggered and showed a sudden increase after 19 days of storage (**Figure 2**), regardless of the packaging atmosphere. Likewise, acetaldehyde content rose markedly (**Figure 3**), with no significant differences among packaging atmospheres.



Figure 3. Acetaldehyde content in packages headspace of Gold cultivar fresh-cut pineapple throughout storage at 5 °C. LO: 12% O<sub>2</sub>, 1% CO<sub>2</sub>. AIR: 20.9% O<sub>2</sub>. HO: 38% O<sub>2</sub>. Each point in the graph is the mean value of four measurements.

Concurrent increase of carbon dioxide, ethanol and acetaldehyde accumulation inside all packages after 15 to 20 days of storage (Figures 1 to 3) confirmed anaerobic respiration reactions of fresh-cut pineapple. Several authors have reported that low concentrations of O<sub>2</sub> and/or high CO<sub>2</sub> promote anaerobic respiration which results in the accumulation of acetaldehyde, ethanol and further increase of carbon dioxide content, which is also an intermediate product of fermentation (3, 4, 20-22). The major function of fermentative metabolism is to allow an alternative production of ATP through substrate phosphorylation, which permits the plant tissue to temporarily survive (20), but such changes can affect flavor and other sensorial attributes and might allow the growth of undesirable anaerobic microorganisms which can be harmful for consumer health. Hence, anaerobiosis is the product response to stress caused by low oxygen or high carbon dioxide atmospheres and/or internal damage of the product. In fact, Pesis (19) suggested that tissue deterioration of overmature fruits may cause an increase in anaerobic respiration because of reduced mitochondrial activity associated with membrane damage and the losses in cells ability to produce enough energy.

Volatile Compounds of Pineapple. Table 1 shows volatile constituents identified and quantified by headspace solid-phase microextraction for Gold cultivar fresh-cut pineapple during 21 days of storage at 5 °C, packed under three initial internal atmospheres (LO, AIR, HO).

Aroma Profile and Major Components. Twenty volatile constituents of Gold cultivar pineapple were detected in Gold cultivar pineapple aroma, for fresh-cut fruits packaged under the three atmospheres studied. Esters accounted for 95% of total volatile compounds emitted at 30 °C, methyl butanoate, methyl 2-methylbutanoate, and methyl hexanoate being the most abundant volatile components (roughly 75% of total volatiles). They were followed by another two esters, methyl 3-(methylthio)propanoate and methyl 2-methylpropanoate, and a furanone, 2,5-dimethyl-4-methoxy-3(2H)-furanone (mesifurane) (Table 1).

Total volatile compounds content of fresh-cut pineapple was larger for fruit pieces packed in AIR atmospheres during the first two weeks of storage, than for LO and HO. In general, it was observed that volatile compounds content reached maximum concentrations during the second week of storage, regardless of the packaging atmosphere, and decreased thereafter. By day 21, volatiles content decreased in all samples but those packed in air showed the lowest levels of the major components (methyl butanoate, methyl 2-methylbutanoate, and methyl hexanoate) and the total volatiles content, suggesting faster product deterioration beyond 14 days of storage.

In contrast, methyl hexanoate, methyl 3-(methylthio)propanoate, and mesifurane emission decreased throughout the 21 days of storage in fruit pieces stored in AIR. Thus, initial package headspace atmosphere affected total content and individual content of volatile compounds in pineapple pieces, as well as their relative composition, since volatiles content varied throughout storage, although the aroma profile constituents were the same along storage.

The largest reduction of volatiles emission, observed in AIR packages during the third week of storage, was concurrent with the carbon dioxide, ethanol and acetaldehyde production increase inside the packages. These observations suggested that anaerobic metabolism speeded up volatile losses and other deteriorative reactions, and confirm the differentiated effect of the initial headspace atmosphere. In fact, Beaulieu and Baldwin (1) indicated that ester formation in apples originates from oxygen-dependent reactions, thus, depletion of that gas could negatively affect production of esters, which could be the case for some pineapple volatiles, since oxygen concentration rapidly decreased.

Comparison among packaging atmosphere treatments showed that volatiles content of the three major volatiles (methyl butanoate, methyl 2-methylbutanoate and methyl hexanoate) was smaller for LO and HO atmospheres on day zero. Differences were attributed to the effect of the packaging procedures, since the sequence of the system used in this study included three steps: vacuum extraction of air from the package headspace, gas mixture flush over the fresh-cut fruit and heat seal of the tray lids. Thus, vacuum pressure used to replace air also produced the physical extraction of some volatiles.

During the following days, the content of the volatiles built up to maximum levels for all packaging conditions, and later depleted sooner for LO and HO than for AIR. Similar results were found by Beaulieu and Baldwin (1), who also reported a temporary increase in ester accumulation in apples during the first days after processing, explained by the product response to wound stress and reduced resistance for volatiles escaping from fruit tissues once the fruit skin is removed. For pineapple, Lamikanra (14) observed significant changes in volatiles content in Gold cultivar pineapple after one day of storage, for thin slices (1-2 mm) cut from damaged fruit flesh close to exposed cut surfaces, demonstrating stress effect due to fresh-cut processing.

It was interesting to notice that, despite the fact that carbon dioxide and oxygen composition in the package headspace of all trays become very similar along the second week of storage, volatile composition varied in different proportions and rates. Volatiles content in fresh-cut pineapple packed in LO and HO atmospheres decreased earlier (during the second week of storage) than those packed in AIR (during the third week), but an abrupt decrease was observed in AIR packages by the 21st day of storage for methyl butanoate, methyl and ethyl 2-methylbutanoate, methyl hexanoate, and mesifurane. Thus, in general, volatiles in fresh-cut pineapple pieces packed in AIR were better withheld through the first two weeks of storage, whereas volatiles content in fruit pieces under LO and HO atmospheres showed important losses from the seventh to the 14th day of storage, with little variation thereafter.

On the other hand, even though only one packaging film and one volume to product ratio were used, it is likely that they contributed to protect losses of volatiles, since volatile emission of fruit pieces was maintained for at least 2 weeks.

Package headspace composition and volatiles content showed little signs of deterioration during the first two weeks of storage, Table 1. Changes in Volatiles Content (µg/kg) of Gold Cultivar Fresh-Cut Pineapple Packed in AIR, LO and HO Atmospheres throughout Storage at 5 °C<sup>a</sup>

		sto	orage time (days)	
volatile compound	0	7	14	21
LO (12% O <sub>2</sub> , 1% CO <sub>2</sub> )				
methyl 2-methylpropanoate	383 <sup>aA</sup>	728 <sup>bB</sup>	495 <sup>aA</sup>	485 <sup>aA</sup>
methyl butanoate	2435 <sup>aA</sup>	3481 <sup>bA</sup>	2687 <sup>aB</sup>	2460 <sup>bB</sup>
methyl 2-methylbutanoate	2105 <sup>aA</sup>	2271 <sup>bA</sup>	2064 <sup>aA</sup>	2161 <sup>abB</sup>
ethyl 2-methylbutanoate	23.0 <sup>aA</sup>	191.7 <sup>св</sup>	46.6 abb	67.7 <sup>DB</sup>
3-methylbutyl acetate	3.4 <sup>an</sup>	55.0 04	7.8 <sup>^</sup>	27.8 abA
methyl 5-hexenoate	1.8 <sup>an</sup>	0.0 aA	2.4 aB	0.7 aA
melnyi hexanoale	101 <sup>aB</sup>	1001 °C	913 °	773 °
elliyi nexanoale	101 500 <sup>aB</sup>	1091 433 <sup>aA</sup>	200 451 <sup>aA</sup>	605 430 aB
limonene	11 9 <sup>aA</sup>	435 12 9 <sup>aA</sup>	451 11 5 <sup>aAB</sup>	439 11.7 <sup>aA</sup>
(Z)-beta-ocimene	3.9 <sup>aA</sup>	4.0 <sup>aA</sup>	3.8 <sup>aA</sup>	3.2 <sup>aA</sup>
2.5-dimethyl-4-methoxy-3(2H)-furanone	357 <sup>aA</sup>	319 <sup>aB</sup>	350 <sup>aC</sup>	345 <sup>aB</sup>
ethyl heptanoate	2.1 <sup>aA</sup>	17.7 <sup>bB</sup>	6.7 <sup>aB</sup>	14.8 <sup>bB</sup>
ethyl 3-(methylthio)propanoate	7.6 <sup>aA</sup>	58.1 <sup>cB</sup>	13.6 <sup>aA</sup>	32.7 <sup>bB</sup>
nonanal	2.2 <sup>aB</sup>	1.7 <sup>aA</sup>	2.8 <sup>aC</sup>	2.8 <sup>aA</sup>
methyl octanoate	36.1 <sup>aA</sup>	58.4 <sup>bA</sup>	44.7 <sup>abB</sup>	61.7 <sup>bC</sup>
methyl (E)-2-octenoate	0.6 ªA	1.3 <sup>DA</sup>	1.0 <sup>abA</sup>	1.4 <sup>DB</sup>
ethyl octanoate	1.7 ab	41.6 bb	16.4 aAb	43.5 bb
ethyl decanoate	0.7 44	7.8	2.1 44	5.8 <sup>bA</sup>
alpha copaene	9.5 **	14.5 °°	11.2 **	21.7 50
total extracted volatile compounds (LO)	7148	9687	7415	7564
AIR (20.9% U <sub>2</sub> )	561 aB	FOO aA	eoo bB	eou aB
methyl butanoato	2112 bB	3135 <sup>bA</sup>	3550 °C	1250 <sup>aA</sup>
methyl 2-methylbutanoate	2464 °B	2276 <sup>bA</sup>	2437 bcB	1034 <sup>aA</sup>
ethyl 2-methylbutanoate	39.4 <sup>aB</sup>	76.4 <sup>bA</sup>	222.7 °C	22.9 ªA
3-methylbutyl acetate	8.1 <sup>aA</sup>	13.8 <sup>aB</sup>	273.4 <sup>bB</sup>	2.9 <sup>aA</sup>
methyl 5-hexenoate	2.2 <sup>aA</sup>	2.3 <sup>aB</sup>	0.0 <sup>aA</sup>	0.8 <sup>aA</sup>
methyl hexanoate	1452 <sup>cB</sup>	1147 <sup>bB</sup>	494 <sup>aA</sup>	536 <sup>aA</sup>
ethyl hexanoate	213 <sup>aC</sup>	648 <sup>bB</sup>	1272 °C	119 <sup>aA</sup>
methyl 3-(methylthio)propanoate	644 <sup>cC</sup>	582 <sup>bcB</sup>	455 <sup>bA</sup>	241 <sup>aA</sup>
limonene	8.9 <sup>ªA</sup>	24.9 <sup>aA</sup>	7.6 <sup>aA</sup>	13.8 <sup>aA</sup>
(Z)-beta-ocimene	7.2 <sup>bA</sup>	2.7 ab	1.3 <sup>aA</sup>	2.6 aA
2,5-dimethyl-4-methoxy-3(2H)-furanone	487 08	367 <sup>bC</sup>	198 <sup>ar</sup>	217 <sup>aA</sup>
ethyl heptanoate	3.5 <sup>an</sup>	12.8 <sup>bB</sup>	12.6 <sup>SO</sup>	4.1 <sup>arc</sup>
ethyl 3-(methylthio)propanoate	13.4 <sup>ab</sup>	31.3 <sup>27</sup>	97.6 °B	10.0
nonanai	1.0	3.3 90 c <sup>bAB</sup>	1.9	1.9 <sup></sup>
methyl (5) 2 actoracto	99.9 0 e aA	00.0 1 7 <sup>bB</sup>	13.9 0.7 <sup>aA</sup>	24.0 0 e aA
ethyl octanoate	0.0 8.8 aC	36.4 <sup>bB</sup>	24.2 bB	0.0 10 1 <sup>aA</sup>
ethyl decanoate	1.1 <sup>aA</sup>	40 bB	6.3 °B	1.7 <sup>aA</sup>
alpha copaene	13.3 <sup>aAB</sup>	24.3 <sup>bA</sup>	11.8 <sup>aA</sup>	13.0 <sup>aA</sup>
total extracted volatile compounds (AIR)	9142	9048	9788	4110
HO (38% O <sub>2</sub> )				
methyl 2-methylpropanoate	606 <sup>bB</sup>	777 <sup>cB</sup>	643 <sup>bB</sup>	463 <sup>aA</sup>
methyl butanoate	2270 <sup>bA</sup>	3363 <sup>cA</sup>	2238 <sup>bA</sup>	1403 <sup>aA</sup>
methyl 2-methylbutanoate	2056 <sup>aA</sup>	2646 <sup>bB</sup>	1990 <sup>aA</sup>	2186 <sup>aB</sup>
ethyl 2-methylbutanoate	16.3 <sup>aA</sup>	99.8 <sup>bA</sup>	12.5 <sup>aA</sup>	83.1 <sup>bB</sup>
3-methylbutyl acetate	8.1 <sup>aA</sup>	10.3 <sup>aA</sup>	10.3 <sup>aA</sup>	20.3 <sup>aA</sup>
methyl 5-hexenoate	1.33 <sup>aA</sup>	1.51 <sup>aAB</sup>	1.33 <sup>aAB</sup>	0.82 <sup>aA</sup>
methyl hexanoate	1197	1427 60	1040 <sup>DB</sup>	638 <sup>aA</sup>
ethyl hexanoate	37.3 an	335.0	30.7 an	515.1 °D
methyl 3-(methylthio)propanoate	312 abr		307 abr	268 da 2
IImonene (Z) hete esimene	16.9 <sup>at</sup>	10.8 <sup>4</sup>	13.1 °	10.5 <sup>art</sup>
25-dimethyl-4-methovy-2(24)-furanono	4.9 210 <sup>CA</sup>	1.9 106 <sup>aA</sup>	3.0 <sup></sup> 212 <sup>CB</sup>	2.4 <sup></sup> 267 <sup>bA</sup>
ethyl hentanoate	1 5 <sup>aA</sup>	α g aA	اد د ۱۹ <sup>aA</sup>	207 Q Q bAB
ethyl 3-(methylthio)propapoate	7.3 <sup>aA</sup>	14 1 <sup>aA</sup>	7 7 <sup>aA</sup>	20 3 pC
nonanal	0.3 <sup>aA</sup>	1.0 <sup>abA</sup>	0.6 <sup>aA</sup>	21.0 21 <sup>bA</sup>
methyl octanoate	34.9 <sup>aA</sup>	100.4 <sup>bB</sup>	30.4 <sup>aB</sup>	43.8 <sup>aB</sup>
methyl (E)-2-octenoate	0.7 <sup>abA</sup>	1.9 <sup>cB</sup>	0.5 <sup>aA</sup>	1.1 <sup>bB</sup>
ethyl octanoate	0.8 <sup>aA</sup>	11.2 <sup>abA</sup>	0.7 <sup>aA</sup>	20.6 <sup>abA</sup>
ethyl decanoate	1.1 <sup>aA</sup>	1.4 <sup>aA</sup>	0.9 <sup>aA</sup>	46 <sup>bA</sup>
alpha copaene	25.9 <sup>aB</sup>	19.5 <sup>aA</sup>	19.7 <sup>aA</sup>	12.9 <sup>aA</sup>
total extracted volatile compounds $(\ensuremath{\text{HO}})$	6916	9376	6664	5979

<sup>a</sup> Values are means of four replicate pineapple samples, for each compound and atmosphere; concentration means along storage with the same lowercase letters are not significantly different (Duncan p < 0.05); likewise, means with the same uppercase letters reveal not significant differences between packaging atmosphere concentration for each specific compound (Duncan p < 0.05).



Figure 4. Odor activity values (OAVs) of volatiles compounds in fresh-cut pineapple (Gold cultivar) stored under LO, AIR, and HO modified atmosphere conditions after 14 days of storage at 5 °C. LO: 12% O<sub>2</sub>, 1% CO<sub>2</sub>. AIR: 20.9% O<sub>2</sub>. HO: 38% O<sub>2</sub>. Numbers around the graph correspond to pineapple volatile compounds: (1) methyl 2-methylpropanoate; (3) methyl butanoate; (6) methyl 2-methylbutanoate; (9) ethyl 2-methylbutanoate; (10) 3-methylbutyl acetate; (13) methyl hexanoate; (14) ethyl hexanoate; (16) methyl 3-(methylthio)propanoate; (17) limonene; (20) 2,5-dimethyl-4-methoxy-3(2H)-furanone; (21) ethyl heptanoate; (22) ethyl 3-(methylthio)-propanoate; (24) nonanal; (25) methyl octanoate.

whereas symptoms of fermentation processes and losses of volatiles were evident during the following days, suggesting 14 days as the maximum storage period for fresh-cut pineapple at 5 °C in AIR atmospheres.

*Most Odor Active Volatiles.* Contribution of volatile compounds to pineapple aroma was determined as odor activity values (OAVs) at the end of the second week of storage (**Figure 4**). The most active volatile compounds in Gold cultivar fresh-cut pineapple were methyl 2-methylbutanoate, ethyl 2-methylbutanoate, ethyl hexanoate, and mesifurane, regardless of the packaging atmosphere. In addition, it was observed that despite the finding that methyl butanoate and methyl hexanoate concentrations in pineapple flesh were high, their contribution to the fruit aroma was much smaller than that of other volatiles.

Odor activity values of volatile compounds in LO, AIR and HO atmospheres showed the same quality profile from a qualitative point of view (**Figure 4**). However, OAVs of volatiles in pineapple samples packaged in AIR were similar to or exceeded those of the fruit packed in LO or HO, for most of the odor active volatiles. In example, OAVs of volatile methyl 2-methylpropanoate, methyl 2-methylbutanoate and mesifurane showed that they have a similar impact on pineapple aroma for all three atmosphere conditions, whereas OAVs of ethyl 2-methylbutanoate, 3-methylbutyl acetate, ethyl hexanoate and ethyl 3-(methylthio)propanoate were larger for fruit pieces packed under AIR than for those under LO and HO atmospheres, indicating larger contribution of such volatiles to fresh-cut pineapple aroma packed in AIR headspace atmosphere on the 14th day of storage.

On the other hand, it should be highlighted that, despite the fact that OAVs are useful to determine relative contribution of volatile compounds to aroma perception, they are based on individual behavior of volatile compounds in water solutions, hence, they do not consider any synergetic effect among odor active volatiles and how aroma perception could be altered by changes in volatile concentrations. In that sense, Ferreira (23)

Table 2. Average Physicochemical and Antioxidant Properties of Nonvolatile Components of Fresh-Cut Pineapple Quality Stored under LO, AIR and HO Atmospheres throughout 21 Days at 5  $^{\circ}C^{a}$ 

quality attribute	LO	AIR	HO
physicochemical properties			
SSC (%)	$13.3\pm0.3^{a}$	$13.2\pm0.3^{a}$	$13.4\pm0.3^{a}$
TA (mg <sub>citricacid</sub> / 100 mg <sub>fw</sub> )	$0.79\pm0.04^{a}$	$0.79\pm0.04^{a}$	$0.77\pm0.02^{a}$
SSC/TA	$17.0\pm0.8^{a}$	$16.7\pm1.0^{a}$	$17.5\pm0.6^{a}$
рН	$3.45\pm0.07^{\:a}$	$3.42\pm0.07^{a}$	$3.45\pm0.08^{a}$
color			
L*	$68.3\pm3.9^{a}$	$67.3\pm4.6^{a}$	$67.4\pm5.7^{a}$
a*	$-3.9\pm0.8^{\rm a}$	$-3.6\pm0.8^{a}$	$-3.3\pm0.9^{a}$
b*	$33.5\pm3.5{}^{a}$	$33.3\pm3.7^{a}$	$32.1\pm4.2^{a}$
antioxidant			
properties			
vitamin C	548 $\pm$ 34 <sup>b</sup>	561 $\pm$ 39 <sup>b</sup>	$488\pm38^{a}$
(mg/100 mg <sub>fw</sub> )			
antioxidant	$59.0\pm4.1$ <sup>a</sup>	$58.9\pm4.3^{a}$	$54.4\pm5.7^{a}$
capacity			
(% DPPH inhibition)			

 $^a$  Means with the same lowercase letters are not significantly different (Duncan p < 0.05). LO: 12% O<sub>2</sub>, 1% CO<sub>2</sub>. AIR: 20.9% O<sub>2</sub>. HO: 38% O<sub>2</sub>.

pointed out that perception of volatiles in complex mixtures such as wines could be affected by alcohols and other volatile compounds, because they affect the solubility of other volatiles and their real contribution to aroma. Some odors can be enhanced while some others can be hidden in a complex mixture, and the mix of volatile compounds can act as an aromatic buffer with little changes in perceptions when one or several constituents' content varies. The above suggests the need to complement our results with sensory evaluations to determine the actual effect of observed changes in volatiles on pineapple aroma perception throughout storage.

Nonvolatile Components of Pineapple. Table 2 shows average physicochemical and antioxidant characteristics of fresh-cut pineapple packed under LO, AIR and HO initial headspace concentrations.

*Physicochemical Parameters.* Soluble solids content (SSC), titratable acidity (TA), pH, the ratio of soluble solids to acidity (SSC/TA) and color parameter  $L^*a^*b^*$  did not show significant changes (p < 0.05) among either packaging atmosphere nor storage period at 5 °C. These results were explained by the fact that pineapple is a nonclimacteric fruit and, as such, shows little changes in its properties, once it is harvested, and because storage at 5 °C slowed down deterioration processes and microbiological growth (*I2*). Soluble solids content was maintained at 13.3  $\pm$  0.3%, titratable acidity at 0.78  $\pm$  0.03 mg<sub>citricacid</sub>/100 mg<sub>fw</sub>, and fruit pH at 3.43  $\pm$  0.08.

Color parameters  $L^*$ ,  $a^*$  and  $b^*$  for Gold cultivar fresh-cut pineapple showed some variability among samples due to fruit heterogeneity, but not significant differences among packaging conditions or storage time. Additionally, fresh-cut fruit did not show any browning symptom throughout the first 21 days of storage, corroborating color stability of this cultivar flesh attributed to absence of PPO (polyphenoloxidase) activity found in previous studies (2). Average  $L^*$ ,  $a^*$  and  $b^*$  for pineapple flesh were kept at 67.7  $\pm$  4.7,  $-3.9 \pm$  0.8, and 33.0  $\pm$  3.8, respectively. Furthermore, it should be highlighted that color stability throughout time and among packaging conditions is a positive attribute for fresh-cut processing, since it can largely contribute to preserve freshness appearance of the finished product.

In spite of little variation of other physical parameters, juice leakage from pineapple pieces significantly increased (p < 0.05)

during storage and changed with the different initial atmospheres (**Figure 5**). Fresh-cut fruits in the trays initially flushed with HO atmosphere did not lose juices during the first 9 days of storage, and showed less juice accumulation throughout storage. In contrast, fresh-cut pineapple in LO packages exhibited the largest juice buildup.

Juice drainage from pineapple pieces can be explained by the physical damage caused during processing. As the fruit shell is removed and further cuts are performed, tissues are injured, cell structure is disrupted and membranes are weakened. These damages reduce internal fluid withholding capacity of fruit tissues, increase the product surface area in contact with the surrounding atmosphere and favor tissues' deterioration during storage, which further reduce tissues' ability to retain juices.

In addition, our results suggested that headspace  $CO_2$  concentrations contribute to increase juice leakage. Elevated concentrations of this gas could have caused a toxic effect on tissues' physiology or at least accelerated them. This effect was also observed by Budu and Joyce (3) in fresh-cut slices of Smooth cayenne cultivar. Our results also agree with those found in previous studies (2), for which juice leakage rapidly increased after 6–8 days of storage, when internal concentration went beyond 20% CO<sub>2</sub>. The use of packages more permeable to CO<sub>2</sub> is suggested to avoid internal atmosphere buildup of high concentrations of this gas.



**Figure 5.** Accumulated juice in fresh-cut pineapple packages throughout storage at 5 °C. LO:  $12\% O_2$ ,  $1\% CO_2$ . AIR:  $20.9\% O_2$ . HO:  $38\% O_2$ . Each point in the graph is the mean value for two fresh-cut pineapple trays.

Antioxidant Characteristics. Vitamin C content of fresh-cut pineapple was very stable throughout the 20 days storage for all packaging conditions, but differences were found among fruits stored in AIR or LO atmospheres and those under HO (**Table 2**). For fresh-cut pineapple pieces stored under AIR and LO headspace atmospheres, the average concentration was nearly  $555 \pm 36$  mg of vitamin C/100 mg<sub>fw</sub>, whereas that under HO atmosphere was significantly lower ( $488 \pm 38$  mg/100 mg<sub>fw</sub>) (p < 0.05).

Lower concentration of vitamin C in fresh-cut pineapple stored under HO atmosphere was explained by larger oxygen headspace concentration and lower carbon dioxide content, which favored vitamin C oxidation, as observed by Soliva-Fortuny et al. (24) and Odriozola-Serrano et al. (25, 26), who found increased vitamin C degradation of fresh-cut pears and tomato slices, for higher oxygen content in the package headspace. The same authors observed very small changes in vitamin C content for tomato slices during 11 days of storage at 5 and 10 °C and over 21 days at 4 °C for slices stored under modified atmosphere packaging (5 kPa of O<sub>2</sub> and 5 kPa of CO<sub>2</sub>), and attributed increased stability to low oxygen concentration. Pineapple is recognized as a good source of vitamin C, thus high content and stability of this vitamin in fresh-cut pineapple are important for consumer acceptability.

Figure 6 shows the effect of packaging conditions on total phenolic compounds (TPC) of fresh-cut pineapple during storage at 5 °C. An increase in TPC was observed during the first days of storage under LO and AIR atmospheres, followed by a steady decrease throughout storage. Initial increase of TPC could be explained by the increase of phenolic compounds produced as a response to injuries occurring during processing, with the aim to repair wound damage and resist microbial invasion (26).

The same authors observed enhanced oxidative stress induced by too low  $O_2$  and high  $CO_2$  concentrations inside tomato slice packages and attributed it to increase of phenylalanine lyase (PAL) activity, which is the key enzyme that uses phenylalanine to synthesize phenolic compounds. In contrast, TPC of fresh-cut pineapple stored under HO atmosphere did not increase during the first storage days, but continually decreased throughout storage. TPC were significantly different in HO atmospheres as compared with LO and AIR atmospheres, explained by larger oxygen concentration in the package headspace during the first two weeks of storage, which could have favor oxidative processes in the fruit.



Figure 6. Total phenolic content changes during storage of fresh-cut pineapple at 5 °C. LO: 12% O<sub>2</sub>, 1% CO<sub>2</sub>. AIR: 20.9% O<sub>2</sub>. HO: 38% O<sub>2</sub>. Each point in the graph is the mean value for two fresh-cut pineapple trays.

Then, passive modified atmosphere packaging (AIR) allowed the preservation of volatile compounds and nonvolatile components in fresh-cut pineapple of the cultivar Gold during storage at 5 °C for at least 14 days of storage and permitted longer withholding of volatile emission and antioxidant attributes than LO and HO atmospheres. The use of an oxygen enriched atmosphere (HO) reduced juice leakage from pineapple pieces, but favored losses in volatile compounds content and antioxidant characteristics and accelerated acetaldehyde production after the second week of storage. Methyl 2-methylbutanoate, ethyl 2-methylbutanoate, 2,5-dimethyl-4-methoxy-3(2H)-furanone and ethyl hexanoate were the most active volatiles in pineapple aroma throughout storage and could be used as quality indicators of fresh-cut pineapple throughout storage. High concentrations of CO<sub>2</sub> promoted volatile losses, juice leakage, and anaerobic respiration.

Vitamin C content and antioxidant capacity did not vary throughout time, but they were better preserved under LO and AIR atmospheres, whereas mechanical properties, color parameters  $L^*$ ,  $a^*$  and  $b^*$ , SSC, TA and pH did not significantly change over time, under any of the packaging conditions.

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