



Quality of guava (*Psidium guajava* L. cv. Pedro Sato) fruit stored in low-O₂ controlled atmospheres is negatively affected by increasing levels of CO₂



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ABSTRACT

Guava is a climacteric fruit, extremely perishable, that has a short shelf life. Controlled atmosphere (CA) and cold storage can be used to extend guava shelf life. However, few studies have been conducted to evaluate the effects of CA storage on guavas, especially with high levels of carbon dioxide (CO₂). Therefore the objective of this study was to evaluate the quality changes of 'Pedro Sato' guava fruit during CA storage with atmospheres containing low concentrations of O₂ and increasing levels of CO₂. For that, 'Pedro Sato' guavas were stored at 12.2 °C for up to 28 days in atmospheres with low oxygen (O₂) concentration (5 kPa) and increasing level of CO₂ (1, 5, 10, 15 or 20 kPa CO₂) in order to evaluate the fruit quality changes. It was possible to identify the relationships among quality variables in response to atmosphere composition and storage duration which were related to modifications due to ripening (ratio SS/TA, TSS, RS, TA), ripening and early CO₂ injury modifications (pH, SS, chromaticity), and CO₂ injury modifications (hue angle, firmness, soluble pectin). A clear CO₂ injury occurred in fruit stored in 5 kPa O₂ + 15 kPa CO₂ and 5 kPa O₂ + 20 kPa CO₂ after 28 days at 12.2 °C, with increasing pH values and soluble pectin content, which were inversely related to fruit firmness. 'Pedro Sato' guavas should be stored in atmospheres with 5 kPa O₂ and no more than 5 kPa CO₂ in order to prevent CO₂ damage. Further investigation is necessary to identify the metabolites responsible for the onset of responses to high CO₂ in guava fruit.

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1. Introduction

Guava is a climacteric fruit (Akamine and Goo, 1979), extremely perishable that has a shelf life of only two days when maintained at room temperature (Pantastico et al., 1979; Carvalho, 1994). Jacomino et al. (2003) reported a longer shelf life, up to six days, when 'Pedro Sato' guavas were stored at 25 °C. This period can be extended to 28 days in cool storage at 8.0 °C (Yamashita and Benassi, 2000). However, as many tropical fruit, guava is sensitive to low temperatures and can be injured by temperatures lower than 10 °C (Bleinroth, 1996; Nakasone and Paull, 1998). Therefore, other

storage methods must be used in order to increase the shelf life of this fruit.

The use of controlled atmosphere (CA) has been recommended to increase the postharvest shelf life of many fruit (Kader, 1986), and few studies have been conducted to evaluate the effects of CA storage on guavas (Broughton and Leong, 1979; Castro and Sigrist, 1988; Singh and Pal, 2008; Teixeira and Durigan, 2010). Kader (2003) recommended atmospheres containing 2–5% oxygen (O₂) and 0–1% carbon dioxide (CO₂) for guavas stored at 5–15 °C. Other recommendations included lower levels of O₂ and higher levels of CO₂, based on the evidence that these fruits do not tolerate CO₂ concentrations higher than 10% (Broughton and Leong, 1979). The beneficial effects of CA with low O₂ and/or high CO₂ concentrations on the postharvest shelf life of guavas include reduction in respiration rates, ethylene production (Singh and Pal, 2008; Teixeira and Durigan, 2010), colour change, and softening (Teixeira and Durigan,

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2010), retention of vitamins, sugars and organic acids, the inhibition of some physiological disorders (Singh and Pal, 2008), and diseases (Teixeira et al., 2007).

Prolonged storage in low O₂ and/or high CO₂ CA can cause adverse effects such as the accumulation of ethanol and acetaldehyde, development of off-odours and off-flavours, failure to ripen after removal from CA storage and development of injuries by low O₂ and/or high CO₂ level(s) (Ke et al., 1991). Physiological disorders caused by low O₂ CA storage conditions are commonly seen in apples (Ke et al., 1991; Golias and Bottcher, 2002) and pears (Ke et al., 1991; Golias and Bottcher, 2002), specifically when O₂ concentrations are below the Pasteur point, which induces anaerobic metabolism and, consequently, the accumulation of acetaldehyde and ethanol (Pesis, 2005). Similarly, damage caused by high CO₂ is characterized primarily by darkening of the interior pulp (Castro et al., 2008), which most likely occurs because of oxidative damage that can be aggravated by low O₂ concentrations (Larrigaudiere et al., 2001; Pesis, 2005). In stressful conditions, hydrogen peroxide (H₂O₂) is produced, and high concentrations of CO₂ inside the cells cause oxidative damage and further H₂O₂ accumulation, which can lead to modifications of the cell membranes (Larrigaudiere et al., 2001). Furthermore, high CO₂ concentrations can cause the accumulation of acetaldehyde and ethanol and a reduction in intracellular pH (Siriphanich and Kader, 1986).

In agreement to Broughton and Leong (1979), who related that guava fruit do not tolerate CO₂ concentrations higher than 10%, Castro and Sigrist (1988) reported symptoms of guava fruit damage caused by high levels of CO₂ in atmospheres with 20% CO₂, after three weeks of storage at 12 °C. However, these studies did not provide the quality modifications of guava fruit under such stressful conditions neither presented the relationships among a set of variables assumed to be responsible for the covariation among the observed variables. Therefore, the objective of this study was to evaluate the quality changes of 'Pedro Sato' guava fruit during CA storage with atmospheres containing low concentrations of O₂ and increasing levels of CO₂.

2. Materials and methods

2.1. Plant material

Guava fruit of the 'Pedro Sato' cultivar were collected in a commercial orchard located at the town of Vista Alegre do Alto, São Paulo (48°21' W and 21°10' S), Brazil. Fruit were harvested at maturity stage 1, according to the recommendation of Azzolini et al. (2004), hue angle (°h) between 117 and 120.

2.2. CA treatments

After selection, 12 guava fruit were placed into a hermetic 1.75-L plastic bucket, each bucket represented one repetition, and there were three repetitions per treatment. These buckets were ventilated with humid air flow balanced with nitrogen (N₂), with a flow rate of 100 mL min⁻¹ at 12.2 ± 0.6 °C, with the following gas composition: (1) 5 kPa O₂, control; (2) 5 kPa O₂ + 1 kPa CO₂; (3) 5 kPa O₂ + 5 kPa CO₂; (4) 5 kPa O₂ + 10 kPa CO₂; (5) 5 kPa O₂ + 15 kPa CO₂ and (6) 5 kPa O₂ + 20 kPa CO₂. The guava fruit were stored in these conditions for 28 days, and every 14 days one group was removed from the CA storage to determine the effect of the CA on the metabolic process.

2.3. Atmospheric control and respiratory activity

To create different atmospheres, it was used two flowboards, constructed according to Claypool and Keefer (1942). This is a classic and highly reliable piece of equipment. Compressed air was

used as the source for O₂, and it was obtained with an air compressor (Shulz, MS 3/30L model, São Paulo, Brazil). The other gases (CO₂ and N₂) were provided by cylinders (White Martins, São Paulo, Brazil). The concentrations of O₂ and CO₂ in all of the mixtures were determined daily by a gas analyser (Dansensor Checkmate 9001, PBI Dansensor, Denmark). The respiratory activity was calculated by an equation obtained from Boyle's and Charles's Law as described by Nakamura et al. (2003).

$$Q = \left(\frac{\Delta C}{100} \right) \times (F \times 60) \times \rho \times (T_0/T)/M$$

where Q = respiratory rate (mg CO₂ kg⁻¹ h⁻¹); ΔC = difference in gas concentration (carbon dioxide) between input and output (%); F = gas flow from sampling chamber (mL min⁻¹); ρ = gas density (g L⁻¹); T₀ = storage temperature (K); T = constant (=273.15); M = sample mass (kg).

Samples from the mixed gases at the input and output of the storage containers (plastic buckets) were taken for the tests, at an interval of 1 h.

The level of carbon dioxide (CO₂) in each sample was quantified by injecting 0.3-mL samples into a gas chromatograph (Finningan, model 9001, Finningan Corporation, San Jose, CA, USA) equipped with stainless steel columns filled with Porapak-N and a molecular sieve (5A), thermal conductivity detectors (150 °C) and flame ionization, using nitrogen as the carrier gas (30 mL min⁻¹). The data was gathered using Borwin software (Borwin version 1.20, JMBS Développements, Le Fontanil, France).

2.4. Quality assessments

2.4.1. Fresh fruit weight loss

This was calculated as a function of the variation of the fruit mass in the different samples, using a semi-analytical scale with precision of 0.01 grams (Marte, model AS 2000, São Paulo, Brazil).

2.4.2. Appearance

Fruit appearance was evaluated according to a grading scale proposed by Teixeira and Durigan (2010), as follow: 5, excellent; 4, very good; 3, acceptable; 2, bad; 1, extremely bad. This evaluation was carried out immediately after the fruits were removed from CA storage (0, 14 and 28 days).

2.4.3. Firmness

Fruit firmness was measured using the Effegi Fruit Tester texturometer (Bishop FT 327, Alfonsine, Italy) and an 8.0 mm flat tip probe. Each fruit was measured twice in the equatorial region, on opposite sides, after the skin was removed; the results were expressed in Newtons (N).

2.4.4. Colour

Outer skin colour was analyzed using a Minolta reflectometer (Model CR-400, Minolta Corp., Osaka, Japan) with an 8 mm viewing aperture, which expressed this parameter according to the system proposed by "L'Eclairage International Commission" (CIE) directly in luminosity (L*), chromaticity and hue angle (McGuire, 1992). Two readings per fruit were performed, on opposite sides and in the equatorial region, and three fruits were used per repetition.

2.4.5. Physicochemical and chemical analyses

Fruit were homogenized and the pulp was used to determine the soluble solids (SS) and total acidity (TA), according to the methods suggested by AOAC (1997 – proc. 920.151 and 932-12, respectively), which allowed the SS/TA ratio to be calculated. Fruit pH was also measured (AOAC, 1997 – proc. 945-27). Four fruits per repetition were processed for the SS, TA and pH analyses. The SS (%) levels were obtained with a digital refractometer (Atago PR 101,

Tokyo, Japan). TA was determined by titrating 10 g pulp, after dilution with 50 mL distilled water, against a 0.1-N NaOH solution using phenolphthalein as an indicator. TA was expressed as a percentage of citric acid. The SS/TA ratio was also calculated. Pulp samples were rapidly frozen at -20°C and subsequently used to determine the total soluble sugars (TSS), reducing sugars (RS), soluble pectin (SP). The frozen pulp (5 g) was mixed with 80 mL of 80% (v/v) ethanol for 1 h in a horizontal shaker. The extract was brought to a volume of 100 mL and filtered with Whatman No. 1 filter paper. One-millilitre aliquots were transferred from the filtered sample to 100 mL volumetric flasks. The levels of TSS were determined by its reaction with anthrone (9,10-dihydro-9-oxo-antraceno), according to the colorimetric analysis proposed by Yemm and Willis (1954). Within the same extract, the RS was determined using 3,5-dinitrosalicylic acid, according to Miller (1959). Soluble pectin was extracted according to procedures described by McCready and McComb (1952) and colorimetrically determined with carbazole (Bitter and Muir, 1962).

2.5. Statistical analyses

2.5.1. Univariate analysis

The experiment was laid out in a completely randomized design (CRD) with a 6×3 factorial plan; that is, six gas concentrations and three sample dates (0, 14 and 28 days), with three repetitions. The data was subjected to an ANOVA and the means were compared using Tukey's test at a $p < 0.05$ confidence level. The PROC MIXED procedure of the SAS (1998) computational system was used for data analysis.

2.5.2. Multivariate analysis – factor analysis

As the postharvest modifications during CA storage is a result of multiple physiological and biochemical processes a factor analysis as used to explore the relationships among the studied variables and treatments (gas concentration). Firstly, the variables were standardized (normal distribution, mean = 0, variance = 1), and secondly the factors were extracted for principal components which were calculated from the correlation matrix among variables, using varimax rotation (Kaiser, 1958). The first factor extracted from that matrix is the linear combination of the original variables, which accounts for as much of the variation contained in the samples as possible. The second factor is the second linear function of the original variables, which accounts for most of the remaining variability, and so on. The coefficients of the factors are used to interpret the relationships among variables, by using the sign and relative size of the coefficients (loadings) as an indication of the weight to be placed upon each variable (Milstein et al., 2005). The effect of the six treatments (gas concentrations), three sampling dates (0, 14 and 28 days) and their cross effect on factor extracted were tested with the General Linear Model (GLM) used as analysis of variance (ANOVA). Differences between levels of the significant main effects (six treatments and sample dates) were tested with the Tukey ($p < 0.05$) multicomparison test of means. The analyses were run using the procedures FACTOR and GLM of the Statistica software (StatSoft, 2004).

3. Results and discussion

The greatest benefit of the storage atmospheres, particularly with low oxygen (5 kPa O_2), was the inhibition or retardation of fruit ripening (Table 1), including the reduction in respiration rate (Fig. 1), retardation of changes in colour ($^{\circ}\text{h}$) (Table 1, Fig. 2), firmness (Fig. 3), and maintenance of sugar levels (Table 2). It was also possible to identify isolated detrimental quality modifications when guavas were stored in high- CO_2 atmospheres, especially 10, 15, and 20 kPa. In these atmospheres occur a decrease in hue angle

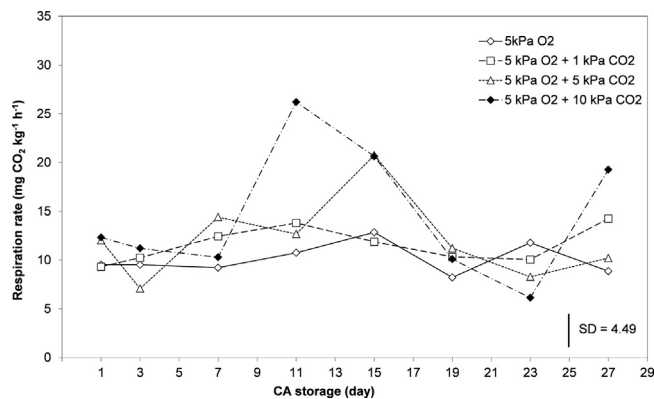


Fig. 1. Respiration rate ($\text{mg CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$) of 'Pedro Sato' guava fruit stored under different atmospheres at 12.2°C for up to 28 days. Vertical bar denote S.E. value.

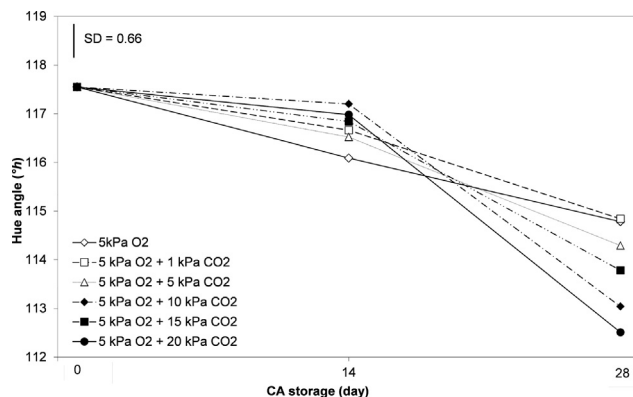


Fig. 2. Hue angle values ($^{\circ}\text{h}$) of 'Pedro Sato' guava fruit stored under different atmospheres at 12.2°C for up to 28 days. Vertical bar denote S.E. value.

($^{\circ}\text{h}$) (Fig. 2), firmness (Fig. 3), reducing sugars content (Table 2), and an increase in soluble pectin (Fig. 4) and pH (Fig. 5).

3.1. Effect of different atmospheres on respiration, appearance and fresh weight loss

The storage of 'Pedro Sato' guavas in atmospheres with 5 kPa O_2 and increasing levels of CO_2 (1, 5, 10, 15 and 20 kPa) at 12.2°C for 28 days did not significantly affected the respiration rates of the fruit (Fig. 1). Guavas maintained in 5 kPa O_2 (control) had the same respiration rates as those maintained in 1, 5 and 10 kPa CO_2 throughout the entire storage period (Fig. 1). This might be due

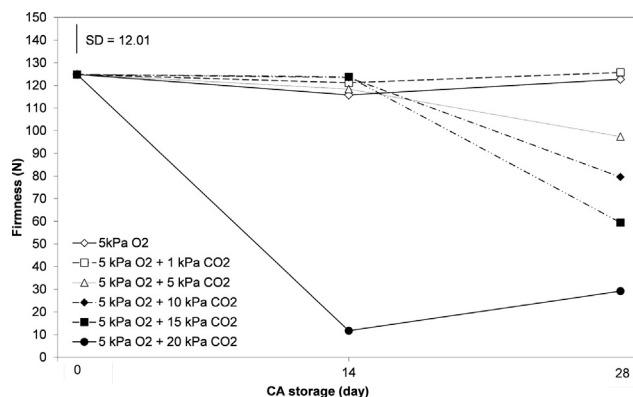


Fig. 3. Firmness (N) of 'Pedro Sato' guava fruit stored under different atmospheres at 12.2°C for up to 28 days. Vertical bar denote S.E. value.

Table 1

Effect of different atmospheres on fresh weight loss (%), colour (lightness and chromaticity) and appearance of 'Pedro Sato' guava fruit after 28 days of storage at 12.2 °C.

| Main factors | Fresh weight loss (%) | Colour | | Appearance ¹ (5 to 1) |
|---|-----------------------|-------------------------------------|--------------|-------------------------------------|
| | | Lightness (<i>L</i> [*]) | Chromaticity | |
| Atmospheres (A) | | | | |
| 5 kPa O ₂ – control | 0.48 | 54.36 | 41.41 | 5.00 |
| 5 kPa O ₂ + 1 kPa CO ₂ | 0.56 | 54.60 | 41.51 | 4.85 |
| 5 kPa O ₂ + 5 kPa CO ₂ | 0.59 | 54.72 | 41.19 | 4.96 |
| 5 kPa O ₂ + 10 kPa CO ₂ | 0.64 | 53.64 | 41.37 | 4.85 |
| 5 kPa O ₂ + 15 kPa CO ₂ | 0.84 | 54.59 | 40.60 | 4.74 |
| 5 kPa O ₂ + 20 kPa CO ₂ | 0.72 | 54.89 | 40.35 | 4.63 |
| CA storage² (B) | | | | |
| 0 | 0.00 c | 54.08 | 39.30 c | 5.00 a |
| 14 | 0.75 b | 54.11 | 40.59 b | 5.00 a |
| 28 | 1.17 a | 55.21 | 43.33 a | 4.52 b |
| Interaction | | | | |
| A × B | NS | NS | NS | NS |

¹ Appearance (5, excellent – 1, extremely bad).² Storage in days.Means within a main effect followed by the same letter in the column are not significant by Tukey's test ($p < 0.05$). NS, interaction not significant.**Table 2**

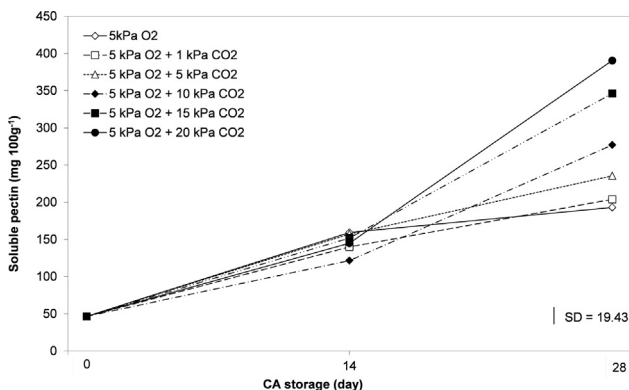
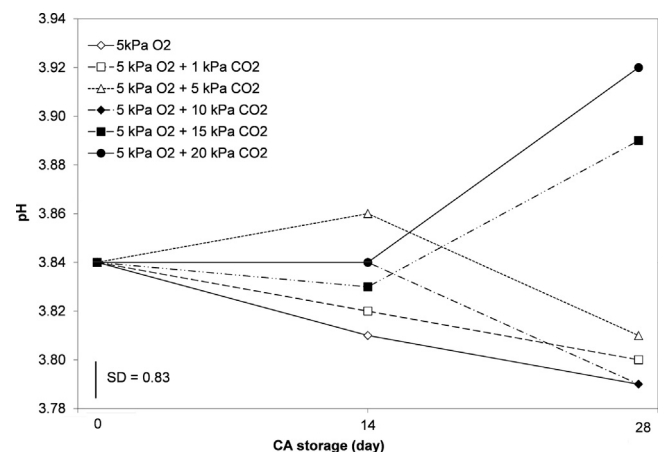
Effect of different atmospheres on titratable acidity (TA), soluble solids content (SS), ratio (SS/TA), total soluble sugar (TSS) and reducing sugar (RS) contents of 'Pedro Sato' guava fruit after 28 days of storage at 12.2 °C.

| Main factors | TA (g 100 g ⁻¹) | SS (%) | SS/AT | TSS (g 100 g ⁻¹) | RS (g 100 g ⁻¹) |
|---|-----------------------------|----------|---------|------------------------------|-----------------------------|
| Atmospheres (A) | | | | | |
| 5 kPa O ₂ – control | 0.54 | 10.30 a | 19.11 | 4.69 a | 3.42 a |
| 5 kPa O ₂ + 1 kPa CO ₂ | 0.54 | 10.03 ab | 18.65 | 4.48 ab | 3.35 ab |
| 5 kPa O ₂ + 5 kPa CO ₂ | 0.55 | 9.73 ab | 17.79 | 4.30 b | 3.15 b |
| 5 kPa O ₂ + 10 kPa CO ₂ | 0.53 | 9.68 ab | 18.16 | 4.42 ab | 3.21 ab |
| 5 kPa O ₂ + 15 kPa CO ₂ | 0.54 | 9.73 ab | 18.15 | 4.29 b | 3.15 b |
| 5 kPa O ₂ + 20 kPa CO ₂ | 0.52 | 9.60 b | 18.50 | 4.51 ab | 3.17 b |
| CA storage¹ (B) | | | | | |
| 0 | 0.48 c | 9.50 b | 19.79 a | 4.71 a | 3.38 a |
| 14 | 0.54 b | 9.88 ab | 18.20 b | 4.43 b | 3.46 a |
| 28 | 0.59 a | 10.16 a | 17.19 c | 4.17 c | 2.89 b |
| Interaction | | | | | |
| A × B | NS | NS | NS | NS | NS |

¹ Storage in days.Means within a main effect followed by the same letter in the column are not significant by Tukey's test ($p < 0.05$). NS, interaction not significant.

to the atmospheres with low O₂ already caused an accentuate reduction in the respiration rate of 'Pedro Sato' guavas (Teixeira and Durigan, 2010). Similar effects were reported by Broughton and Leong (1979), Kader (1986), Castro and Sigrist (1988), Pal and Buescher (1993), Singh and Pal (2008) and Teixeira and Durigan (2010) in guavas stored in CA systems and in other fruit such as the cherimoya (Palma et al., 1993), apple (Saquet and Streif, 2002) and 'Tommy Atkins' mangos (Bender et al., 2000). It is worth to note

that data from the treatments of 5 kPa O₂ + 15 kPa CO₂ and 5 kPa O₂ + 20 kPa CO₂ were not presented because it was not possible to measure the respiration rates of the fruit in these atmospheres, likely due to the saturation of the chromatograph column with higher levels of CO₂ (15 and 20 kPa).

**Fig. 4.** Soluble pectin content (mg 100 g⁻¹) of 'Pedro Sato' guava fruit stored under different atmospheres at 12.2 °C for up to 28 days. Vertical bar denote S.E. value.**Fig. 5.** Potential of hydrogen (pH) of 'Pedro Sato' guava fruit stored under different atmospheres at 12.2 °C for up to 28 days. Vertical bar denote S.E. value.

As atmospheres were humidified by bubbling the air flow into a glass jar containing water, fresh fruit weight loss (FWL) was very low, only 1.17% after 28 days of CA storage (Table 1). This procedure helped to maintain the quality of the fruit, particularly appearance (Table 1). There was no significant difference between treatments, and a slight deterioration in appearance due to a low incidence of fruit rot was only noticed after 28 days of storage. However, the fruit were considered “excellent” or “very good” throughout the storage period.

3.2. Effect of different atmospheres on colour and firmness

The different CA conditions did not have a significant effect on luminosity (L^*) or chromaticity on guava fruit (Table 1). However, the duration in storage significantly affected the chromaticity ($p < 0.05$), with an increase of 39.30–43.33 from the beginning to the end of storage (28 days), Table 1.

On the other hand, the hue angles ($^{\circ}h$) and firmness presented significant interaction between the different atmospheres and storage duration (Figs. 2 and 3). The fruit kept in 5 kPa O_2 (control) and low levels of CO_2 (5 kPa O_2 + 1 kPa CO_2 and 5 kPa O_2 + 5 kPa CO_2) showed smaller $^{\circ}h$ reductions ($p < 0.05$) (greener fruit) than those that were stored in higher CO_2 levels (Fig. 2). This effect was most evident on the 28th day of storage, when there was a noticeable reduction in $^{\circ}h$ as CO_2 concentrations increased (Fig. 2). Similarly, there was a reduction in fruit firmness with increasing CO_2 levels, but the fruit maintained in 5 kPa O_2 (control) and 5 kPa O_2 + 1 kPa CO_2 remained firmer than those in the other treatments ($p < 0.05$), with average values of 122.70 N and 125.74 N, respectively, at the end of storage. It is worth to point out that there was a reduction in the firmness of the fruit during storage, especially in those stored in 5 kPa O_2 + 20 kPa CO_2 , which softened significantly after 14 days in this condition (Fig. 3).

3.3. Effect of different atmospheres on titratable acidity, soluble solids content, ratio (SS/TA), total soluble sugar and reducing sugar

The different atmospheres did not affect the titratable acidity (TA) contents and SS/TA ratios (Table 2), but these were affected by the storage duration. There was an increase in TA level (0.48–0.59 g 100 g⁻¹) and a decrease in SS/TA ratio from 19.79 to 17.19, from the beginning to the end of CA storage (28 days), respectively (Table 2). The different CA conditions significantly affected the levels of soluble solids (SS), total soluble sugars (TSS) and reducing sugars (RS) (Table 2). The fruit stored in 5 kPa O_2 (control) had higher levels of SS, TSS and RS than those stored in 5 kPa O_2 + 20 kPa CO_2 , and these levels did not vary significantly among the other atmospheres (Table 2). Storage period also had a significant effect on these parameters, with an increase in SS levels from 9.5% to 10.2% at the beginning and end of storage, respectively (Table 2). However, TSS and RS levels dropped during the same period, from 4.71 to 4.17 g 100 g⁻¹ and from 3.31 to 2.89 g 100 g⁻¹, respectively (Table 2).

3.4. Effect of different atmospheres on soluble pectin and pH

There was a significant interaction ($p < 0.01$) between atmospheres and storage duration for soluble pectin contents (Fig. 4) and the pH (Fig. 5). The soluble pectin (SP) content increased during CA storage, and the increases were noticeable after 14 days under these conditions (Fig. 4). Guavas stored in 5 kPa O_2 (control) and 5 kPa O_2 + 1 kPa CO_2 had lower soluble pectin levels than those stored in 5 kPa O_2 + 10 kPa CO_2 , 5 kPa O_2 + 15 kPa CO_2 and 5 kPa O_2 + 20 kPa CO_2 (Fig. 4). The pH of the fruit remained practically unaltered until the 14th day of storage in all treatments. However, at the end of 28

Table 3

Results of factor analysis, ANOVA and Tukey mean multicomparisons of ‘Pedro Sato’ guava fruit stored under different controlled atmospheres conditions at 12.2 °C for up to 28 days.

| Variables | Factor 1 | Factor 2 | Factor 3 |
|---|-------------------------|--|-----------------------------|
| Ratio (SS/TA) | 0.94^a | -0.04 | 0.20 |
| Total soluble sugar (TSS) | 0.91 | 0.20 | 0.21 |
| Reducing sugars (RS) | 0.73 | 0.28 | 0.47 |
| Titratable acidity (TA) | -0.72 | 0.55 | -0.35 |
| Ph | -0.12 | -0.88 | -0.26 |
| Soluble solids (SS) | 0.16 | 0.84 | -0.31 |
| Chromaticity | -0.38 | 0.61 | -0.58 |
| Hue angle | 0.36 | -0.28 | 0.86 |
| Firmness | 0.11 | 0.17 | 0.95 |
| Soluble pectin (SP) | -0.39 | 0.15 | -0.87 |
| Variance explained (%) | 32 | 24 | 33 |
| Interpretation | Ripening | Ripening and early CO_2 injury modifications | CO_2 injury modifications |
| ANOVA test | (SS-Sig.) | (SS-Sig.) | (SS-Sig.) |
| Atmospheres (A) | 5.89 ^{NS} | 13.14 ^{**} | 5.46 [*] |
| CA storage (B) | 20.11 ^{**} | 8.95 ^{**} | 23.97 ^{**} |
| Interaction A × B | 7.56 ^{NS} | 11.89 [*] | 9.65 ^{**} |
| Mean multicomparisons by atmospheres (A) ^b | | | |
| 5 kPa O_2 – control | a | a | A |
| 5 kPa O_2 + 1 kPa CO_2 | a | ab | A |
| 5 kPa O_2 + 5 kPa CO_2 | a | abc | A |
| 5 kPa O_2 + 10 kPa CO_2 | a | abc | Ab |
| 5 kPa O_2 + 15 kPa CO_2 | a | bc | Ab |
| 5 kPa O_2 + 20 kPa CO_2 | a | c | B |
| Mean multicomparisons by CA storage (B) | | | |
| 0 | a | a | A |
| 14 | b | b | A |
| 28 | c | b | B |

^a Factor coefficients in bold were used for interpretation. SS – total sums squares from ANOVA. Sig – significance levels: ** significant at $p < 0.01$; * significant at $p < 0.05$, ^{NS} not significant.

^b Mean multicomparisons: values followed by the same letter in the column are not significant by Tukey’s test ($p < 0.05$).

days of CA storage, the guavas stored in 5 kPa O_2 + 15 kPa CO_2 and 5 kPa O_2 + 20 kPa CO_2 had a higher pH than the others (Fig. 5).

3.5. Relationship among quality variables

The coefficients of the factor analysis performed on ‘Pedro Sato’ guava fruit stored under different CA, and also the outcome of the ANOVA test, and the mean multicomparison test is presented in Table 3. The first three factors explained 89% of the original data variance.

Factor analysis was used in order to understand the relationships among the studied quality variables in terms of a limited number of new variables (factors) and these factors assumed to be responsible for the covariation among the observed variables (Milstein et al., 2005).

Factor 1 contributed to explain 32% of the data variability and it also showed a positive correlation with some variables that indicate guava ripening (ratio SS/TA, total soluble sugar – TSS, and reducing sugars – RS), and these variables correlated negatively with titratable acidity – TA (Table 3). The ANOVA applied in the factor 1 (ripening) showed statistical significance for storage period, with ripening increasing during the 28 days period (Table 3).

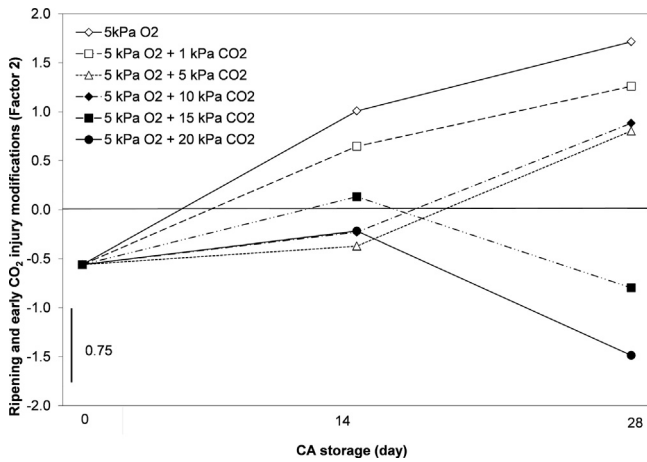


Fig. 6. ANOVA results for Factor 2 showing the interaction between atmospheres and storage period of 'Pedro Sato' guava fruit stored under different atmospheres at 12.2 °C for up to 28 days.

The ripening Factor 1 reflected two different processes that were negatively correlated with storage period (Table 3). On the one hand, TSS, RS and ratio SS/TA decreased during CA storage; on the other hand, TA increased significantly during storage ($p < 0.05$), which are commonly observed during guava storage. Although sugar content (TSS and RS) had a reduction during CA storage the magnitude of this decrease is not meaningful, 4.71–4.17 g 100 g⁻¹, and 3.38–2.89 g 100 g⁻¹, for TSS and RS, respectively (Tables 2 and 3). However, the increase in TA is commonly reported during guava storage. Mercado Silva et al. (1998) reported increases in TA until the seventh day of storage at 25 °C for 'Media China' guava. For 'Pedro Sato' cultivar TA usually increases during storage even when fruit at different maturity stages are stored (Azzolini et al., 2004). Therefore, the reduction in ratio SS/TA reflected the increase in TA, which is normally reported during guava ripening.

The second factor (Factor 2), named ripening and early CO₂ injury modification, explained 24% of the data variability, showing that pH value correlated negatively with soluble solids (SS) and chromaticity (Table 3). The ANOVA applied to Factor 2 showed significant interaction ($p < 0.05$) between atmospheres and storage duration (Table 3). The atmospheres with 5 kPa O₂ + 15 kPa CO₂, (treatment 5) and 5 kPa O₂ + 20 kPa CO₂, (treatment 6) performed differently specially after 28 days of storage (Fig. 6).

The Factor 2, named ripening and early CO₂ injury modification, reflects the importance of fruit exposition to stressful conditions during storage with a significant interaction ($p < 0.05$) between atmospheres and storage duration (Table 3). Fruit stored in 5 kPa O₂ + 15 kPa CO₂ or 5 kPa O₂ + 20 kPa CO₂ presented metabolic changes related to accelerated metabolism and CO₂ injury (Fig. 6). The early CO₂ injury modifications can be related to the increase in pH values of fruit stored in 5 kPa O₂ + 15 kPa CO₂ and 5 kPa O₂ + 20 kPa CO₂, mainly after 14 day of storage (Fig. 5). Siriphanich and Kader (1986) reported that high concentrations of CO₂ might lead to a lower pH, this was not observed here, though. The ripening process continued and it was related to SS content increases during CA storage (Table 2) as commonly reported during guavas storage (Carvalho, 1994; Mercado Silva et al., 1998; Bashir and Abu-Goukh, 2003).

The Factor 3 explained 33% of the data variability (Table 3). This factor presented a negative correlation between soluble pectin (SP) with hue angle (°h) and firmness (Table 3). The ANOVA applied to factor 3 had a significant interaction ($p < 0.01$) between atmospheres and storage duration (Table 3). This factor was named CO₂ injury modification due to the modifications resulted from the

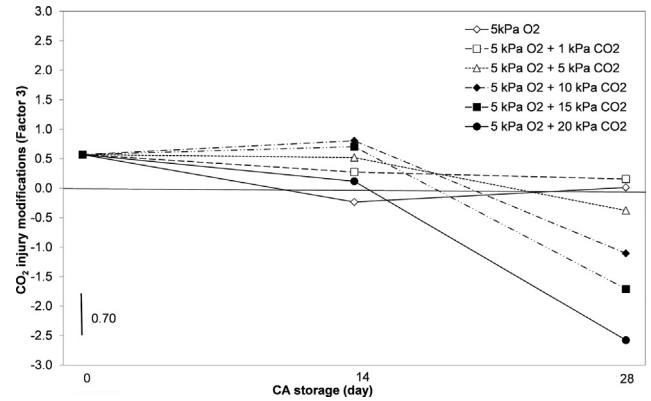


Fig. 7. ANOVA results for Factor 3 showing the interaction between atmosphere and storage period of 'Pedro Sato' guava fruit stored under different atmospheres at 12.2 °C for up to 28 days.

atmospheres, mainly with high levels of CO₂ (Fig. 7), which were characterized by increasing SP contents (Fig. 4) and reduction in fruit firmness (Fig. 3).

The ANOVA applied to Factor 3 had a significant interaction ($p < 0.01$) between atmospheres and storage duration (Table 3). The CO₂ injury modifications Factor 3 showed strong differences between treatments, mainly between fruit stored in 5 kPa O₂ + 15 kPa CO₂ and 5 kPa O₂ + 20 kPa CO₂ (Fig. 7), which were associated with the sharp softening modifications. There was an increase in softening with increasing CO₂ levels, which features the development of a physiological disorder caused by high CO₂, mainly at levels above 10 kPa CO₂ after 14 days of storage (Fig. 3). The decrease in fruit firmness was related to pectic compound modifications that are responsible for the integrity of the cell wall (Tucker, 1993). Soluble pectin (SP) content increased as CO₂ levels increased, becoming more evident at the end of storage (28 days), Fig. 4. Carvalho (1994) and Abu-Bakr et al. (2003) reported decreases in total pectin levels during guava ripening and consequent increase in soluble pectin causing pronounced fruit softening. Therefore, the lower levels of soluble pectin in guavas stored in 5 kPa O₂ (control) and 5 kPa O₂ + 1 kPa CO₂ can be due to the delay in ripening. However, it is more likely that these fruit have not suffered damage from high levels of CO₂, which accelerates the ripening process. In addition, the stress caused by CO₂ and resulting high levels of H₂O₂ might have affected the cell membrane structure, and membrane leakage may lead to the contact of cytoplasmic enzymes such as polygalacturonase (PG, EC 3.2.1.15) and pectolyase (PL, EC 4.2.2.2) with the cell wall, causing the depolymerization of pectin. Eventually the leakage of the membranes may lead to cell death (Castro et al., 2008).

The Factor 3 also showed that hue angle (°h) was positively correlated with firmness and negatively correlated with PS (Table 3). As softening, the fruit colour modifications is triggered by ethylene (Tucker, 1993; Wills et al., 1998), and although atmospheric concentrations of CO₂ can contribute to reduce produce's sensitivity to ethylene, mainly at CO₂ levels higher than 1 kPa (Kader, 1986), the increasing levels of CO₂ did not show a synergistic effect with the oxygen (5 kPa O₂) to control those processes. Fruit stored in 5 kPa O₂ (control) and low CO₂ levels (5 kPa O₂ + 1 kPa, and 5 kPa O₂ + 5 kPa CO₂) showed less reductions in hue angle ($p < 0.05$) than those stored in atmospheres richer in CO₂ (Fig. 2). This effect was more evident at the end of storage (28 days), when a gradual decrease in hue angle was observed as CO₂ concentrations increased (Fig. 2). The observed hue angle values were very similar to those reported by Azzolini et al. (2004) in fully ripe (yellow) 'Pedro Sato' guavas. Probably in levels higher than 5 kPa CO₂ occurred CO₂ injury which promoted increases in the respiration rate and possibly ethylene

production. Because colour change in the outer skin depends on ethylene action, once it is present, this hormone triggers the ripening process and, consequently, the expression of many enzymes involved in chlorophyll breakdown and carotenoid synthesis (Wills et al., 1998). Therefore, the atmospheres containing 5 kPa O₂ (control), 5 kPa O₂ + 1 kPa CO₂ and 5 kPa O₂ + 5 kPa CO₂ were more efficient to maintain colour by delaying the ripening process and/or by not showing damage from high CO₂ concentrations.

4. Conclusions

The quality of 'Pedro Sato' guava fruit was affected by the storage in 5 kPa O₂ and CO₂ levels above 5 kPa (10, 15, and 20 kPa). The increase in CO₂ concentration negatively affected fruit quality by accelerating changes in colour ($^{\circ}h$) and firmness, and increasing the solubilization of pectic compounds.

A clear CO₂ injury happened in fruit stored in 5 kPa O₂ + 15 kPa CO₂ and 5 kPa O₂ + 20 kPa CO₂ after 28 days at 12.2 °C, with increasing pH values and soluble pectin content, which were inversely related to fruit firmness.

'Pedro Sato' guava fruit can be satisfactorily stored in atmospheres containing 5 kPa O₂ and CO₂ up to 5 kPa for 28 days at 12.2 °C and ~95% RU.

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