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# Quality of 'Oso Grande' strawberries is affected by O<sub>2</sub>, CO<sub>2</sub> and N<sub>2</sub>O concentrations during controlled atmosphere storage

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**ABSTRACT:** Consumers around the world appreciate strawberries for their taste. They have low calories and high concentrations of soluble fibers, vitamin C and flavonoids. This paper verified the combined effect of O<sub>2</sub>, CO<sub>2</sub>, and N<sub>2</sub>O levels on 'Oso Grande' strawberries stored at 10° C, under controlled atmosphere (CA). Five different gas mixtures were used: 0.03 kPa CO<sub>2</sub> + 20 kPa O<sub>2</sub>, 80 kPa N<sub>2</sub>O + 20 kPa O<sub>2</sub>, 90 kPa O<sub>2</sub> + 10 kPa N<sub>2</sub>, 60 kPa O<sub>2</sub> + 40 kPa CO<sub>2</sub>, and 20 kPa O<sub>2</sub> + 20 kPa CO<sub>2</sub> + 60 kPa N<sub>2</sub>O. The lowest incidence of postharvest decay was observed with treatment 20 kPa O<sub>2</sub> + 20 kPa CO<sub>2</sub> + 60 kPa N<sub>2</sub>O, followed by 80 kPa N<sub>2</sub>O + 20 kPa O<sub>2</sub> and 90 kPa O<sub>2</sub> + 10 kPa N<sub>2</sub>. The treatment 60 kPa O<sub>2</sub> + 40 kPa CO<sub>2</sub> induced an increase in the production of acetaldehyde and ethanol, and these levels were considered inadequate

for human consumption. The first factor, named senescence, displayed a positive correlation with soluble solids, luminosity, hue angle, firmness and incidence of decay. The second factor, named CA-induced injury, showed that total acidity correlated negatively with ethanol and acetaldehyde levels. Hierarchical cluster analysis indicated that strawberries stored under 80 kPa N<sub>2</sub>O + 20 kPa O<sub>2</sub> for 14 days more closely resembled the quality of fresh fruit at the moment of harvest. 'Oso Grande' strawberries stored at 10 °C under 80 kPa N<sub>2</sub>O + 20 kPa O<sub>2</sub> and 90 kPa O<sub>2</sub> + 10 kPa N<sub>2</sub> were in better conditions, with no metabolic alterations, showing that these are the ideal storage conditions.

**Key words:** *Fragaria x ananassa* Duch, acetaldehyde, ethanol, multivariate analysis.

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## INTRODUCTION

Strawberry has a postharvest life of only two days at room temperature that represents an obstacle to long-distance distribution (Malgarim et al., 2006). Therefore, different techniques have been used to extend strawberry's shelf life, including modified (MAP) or controlled atmosphere (CA) (Cunha Junior et al., 2013). Strawberry has a good tolerance to high  $\text{CO}_2$  storage, which extends postharvest life, reducing the incidence of disease and maintaining fruit firmness. In fact, the benefits of CA in extending the storage life of strawberries have been long well documented (Zhang and Watkins, 2005; Cunha Junior et al., 2013).

Low  $\text{O}_2$  in association or not with high  $\text{CO}_2$  levels can also extend strawberry shelf life (Holcroft and Kader, 1999). On the other hand, high  $\text{O}_2$  concentrations have also been shown to extend the postharvest life of fruit and vegetables. An atmospheric  $\text{O}_2$  pressure of 70 kPa has efficiently reduced respiratory rates as well as the growth of bacteria and fungi during strawberry storage (Escalona et al., 2006). Storage by means of under CA also allows the use of gases with fungicidal effect, such as nitrous oxide ( $\text{N}_2\text{O}$ ) which has been shown to inhibit disease and ethylene production in fruit (Qadir and Hashinaga, 2001).

Many results can be found about strawberry responses to CA storage with different  $\text{O}_2$ ,  $\text{CO}_2$ , and  $\text{N}_2\text{O}$  levels (Escalona et al., 2006). Despite this, there is not a complete recommendation for 'Oso Grande' strawberry an UC Davis variety that represents 80% of the Brazilian production.

In separate studies, we have reported a better 'Oso Grande' strawberry quality when the fruit was stored in different gases. Regarding  $\text{O}_2$  levels, 'Oso Grande' strawberries were stored at 1, 3, 20, 60 and 90 kPa  $\text{O}_2$  at 10 °C. Consequently, best fruit quality was obtained in 60 and 90 kPa atmospheres for up to 8 days because of low decay development and better visual appearance (Cunha Junior et al., 2011).

The  $\text{CO}_2$  levels were also tested, and atmospheres with 0.03, 10, 20, 40 and 80 kPa  $\text{CO}_2$  combined with 20 kPa  $\text{O}_2$  were used to store 'Oso Grande' strawberries at 10 °C. The  $\text{CO}_2$  levels of 20 and 40 kPa maintained the fruit quality for up to 8 days. On the other hand, fruit stored at 80 kPa  $\text{CO}_2$  were excellent in appearance due to decay control development, but presented elevate production of acetaldehyde and ethanol in consequence of the onset of fermentative respiration (Cunha Junior et al., 2012).

$\text{N}_2\text{O}$  was also test as its similarity to  $\text{CO}_2$  might be pertinent to control the ethylene production in CA storage. Therefore, 'Oso Grande' strawberries were stored at 10 °C in atmospheres containing 10, 30, 60 and 80 kPa of  $\text{N}_2\text{O}$  combined with 21 kPa  $\text{O}_2$ . It was possible to get 10 days shelf live when the fruits were stored in 60 kPa and 80 kPa  $\text{N}_2\text{O}$  due to reduced decay incidence and respiration rate (Cunha Junior et al., 2013).

The objective of this study was to verify the combined effect of  $\text{O}_2$ ,  $\text{CO}_2$ , and  $\text{N}_2\text{O}$  levels during 'Oso Grande' strawberries stored at 10 °C, under controlled atmosphere (CA). It was intended as a result of the satisfactory isolated effects of  $\text{O}_2$ ,  $\text{CO}_2$ , and  $\text{N}_2\text{O}$  levels to maintain 'Oso Grande' strawberry fruit quality and the lack of information regarding the association of these gases during CA storage.

## MATERIALS AND METHODS

### Plant material

'Oso Grande' strawberries were obtained from a commercial grower in Valinhos, São Paulo State, Brazil (lat 22° 58' S, long 16° 59' W and 660 m of altitude). Fruit were harvested when 50% to 75% of the epidermis had a bright red color, as recommended by Cunha Junior et al. (2013).

After harvest, strawberries were sorted and air cooled in a cold room without forced air circulation at 10 °C  $\pm$  1 °C and 95%  $\pm$  2% of relative humidity (RH), for 12 hours. After this initial period, fruit were placed in hermetic, translucent plastic boxes (Sanremo® 960, 8.6 L) holding 1.2 kg of strawberry each, at 10 °C  $\pm$  1 °C and 97%  $\pm$  2% RH, for up to 14 days. Each of these mini-chambers was evaluated every two days.

### CA treatments: $\text{O}_2$ , $\text{CO}_2$ , and $\text{N}_2\text{O}$ mixtures

Storage under CA was conducted with five different concentrations of  $\text{O}_2$ ,  $\text{CO}_2$ ,  $\text{N}_2\text{O}$ , balanced with nitrogen ( $\text{N}_2$ ), as such: (control atmosphere) – 0.03 kPa of  $\text{CO}_2$ , 20 kPa of  $\text{O}_2$ ; 80 kPa of  $\text{N}_2\text{O}$ , 20 kPa of  $\text{O}_2$ ; 90 kPa of  $\text{O}_2$ , 10 kPa of  $\text{N}_2$ ; 60 kPa of  $\text{O}_2$ , 40 kPa de  $\text{CO}_2$ ; and 20 kPa of  $\text{O}_2$ , 20 kPa of  $\text{CO}_2$ , 60 kPa of  $\text{N}_2\text{O}$ .

The  $\text{O}_2$ ,  $\text{CO}_2$  and  $\text{N}_2\text{O}$  concentrations were defined based on our previous studies (Cunha Junior et al., 2011; Cunha Junior et al., 2012; Cunha Junior et al., 2013). Gases were obtained from industrial, compressed gas cylinders and the gas mixtures were prepared with the aid of a flow-board,

adapted from Calbo (1989) and then applied into the mini-chambers at a continuous flow rate of  $0.9 \text{ L}\cdot\text{s}^{-1}$ . Atmospheric composition of each mini-chamber was assessed daily with a gas analyzer (Dansensor, model Checkmate 9001, Denmark).

## Quality assessments

### Postharvest decay

The incidence of postharvest decay was determined from 160 strawberries per treatment per sampling day. Fruit that presented lesions larger than  $25 \text{ mm}^2$  and caused by *Rhizopus* spp. and/ or *Colletotrichum* spp. were counted as affected by decay. Results were expressed as percentages transformed to arcsine (Nishijima et al., 1992).

### Firmness

Flesh firmness was determined with the aid of a digital penetrometer model Sammar 53200 (TR Turoni, Italy) with a penetration tip of 6 mm in diameter. Results were expressed in Newton (N). An analysis per fruit was performed on the surfaces of the strawberries, 160 fruit per treatment were used (Cunha Junior et al., 2013).

### Color

External color was determined with a colorimeter (Minolta CR-300), and the results were expressed in luminosity ( $L^*$ ), hue angle ( $^{\circ}\text{h}$ ) and chromaticity (Chr), as proposed by McGuire (1992). It was used 160 fruit per treatment for this analysis, and two readings were performed on opposite sides of each fruit.

### Physical-chemical analysis

Strawberries were homogenized and the pulp was used to determine the soluble solids content (SSC) and total acidity (TA), according to the methods proposed by A.O.A.C. (1997 - proc. 920.151 and 932-12, respectively). The SSC (%) 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 in gram of citric acid per 100 g of sample (%). Ascorbic acid (AA) content was determined as described by Strohecker and Henning (1967), and the results were

expressed as mg equivalents of AA per 100 g of sample. Four repetitions were conducted with 40 strawberries each.

### Acetaldehyde and ethanol levels

Mashed strawberry pulp samples were weighed 1 g and they were collected and stored in hermetic 40 mL flasks at  $-18^{\circ}\text{C}$ . Acetaldehyde and ethanol content was determined with a gas chromatograph (GC Trade 2000 model Thermo Finning, with flame ionization detector (FID) and with a Poropak N column. The results were expressed as gram of acetaldehyde or ethanol per 100 g of plant material (%), as described by Cunha Junior et al., (2013). Four repetitions were performed and each consisted of 40 fruit of strawberries that were mashed together.

## Statistical analysis

### Univariate analysis

The experiment followed a completely randomized design (CRD) in a  $5 \times 8$  factorial, which consisted of five gas mixtures ( $0.03 \text{ kPa CO}_2 + 20 \text{ kPa O}_2$ ;  $80 \text{ kPa N}_2\text{O} + 20 \text{ kPa O}_2$ ;  $90 \text{ kPa O}_2 + 10 \text{ kPa N}_2$ ;  $60 \text{ kPa O}_2 + 40 \text{ kPa CO}_2$ ; and  $20 \text{ kPa O}_2 + 20 \text{ kPa CO}_2 + 60 \text{ kPa N}_2\text{O}$ ) and eight storage periods (0, 2, 4, 6, 8, 10, 12 and 14 days). Data were submitted to analysis of variance (ANOVA) and means test among the treatments (Tukey's test at 5% probability). The statistical analysis was carried out using Statistica software, version 7 (StatSoft, 2004).

## Multivariate analysis

### Factor analysis

The data set with all variables was reduced to its mean and it was standardized so that each variable had a zero mean and unit variance (Hartigan, 1975). The main physiological and biochemical processes contained in the measured variables were identified by means of factor analysis (Milstein, 1993). Factors were extracted by the principal component method and they were calculated from the variable correlation matrix using the Varimax rotation (Kaiser, 1958).

The first factor extracted from the matrix was the linear combination of original variables, which represented the maximum possible amount of variance contained in the samples (Milstein et al., 2005).

The second factor was the second linear function of the original variables, representing most of the remaining



variance, and so on. Factor loadings were used to interpret the relationship among variables, using the sign and relative size of loadings as an indication of the weight of each variable (Milstein et al., 2005).

### Principal component analysis (PCA)

PCA was used to identify which treatments contributed to the onset of physiological and biochemical processes. PCA generates orthogonal latent variables centered in a region with the highest concentration of variability. Eigenvalues and eigenvectors (principal components) were extracted from the data covariance matrix in accordance with the Kaiser criteria. In this way, eigenvalues above one were considered, which generated components with a relevant amount of information from the original data (Kaiser, 1958).

### Hierarchical cluster analysis (HCA)

HCA was conducted for all treatments and for each evaluation day, using as similarity coefficient the Euclidian Measure of Dissimilarity, and the Ward Algorithm as clustering strategy (Hair et al., 2005). Thus, we found the storage conditions that better preserved strawberries.

## RESULTS AND DISCUSSION

### Univariate analysis

Under 20 kPa O<sub>2</sub> + 20 kPa CO<sub>2</sub> + 60 kPa N<sub>2</sub>O strawberries displayed the lowest incidence of postharvest decay on

day 14 (5.7%,  $p < 0.05$ ), followed by strawberries stored in 80 kPa N<sub>2</sub>O + 20 kPa O<sub>2</sub> and 90 kPa O<sub>2</sub> + 10 kPa N<sub>2</sub>. Their decay incidence rates were 19% and 23%, respectively. Strawberries stored at 60 kPa O<sub>2</sub> + 40 kPa CO<sub>2</sub> did not develop decay until day 8, however the fruit produced a strong fermentation odor (Table 1).

The strong odor produced of strawberries stored in 60 kPa O<sub>2</sub> + 40 kPa CO<sub>2</sub> may be explained by high levels of ethanol and acetaldehyde. Initial values (day 0) were 0.0004% of acetaldehyde and 0.0001% of ethanol, and after 8 days under 60 kPa O<sub>2</sub> + 40 kPa CO<sub>2</sub>, levels climbed to  $0.0548 \pm 0.0016\%$  and  $0.0424 \pm 0.0021\%$ , respectively (Figs. 1a and 1b). These modifications made the strawberries inadequate for consumption. In addition, potential benefits of 60 kPa O<sub>2</sub> + 40 kPa CO<sub>2</sub> in controlling postharvest decay were outweighed by the negative effects on flavor and odor.

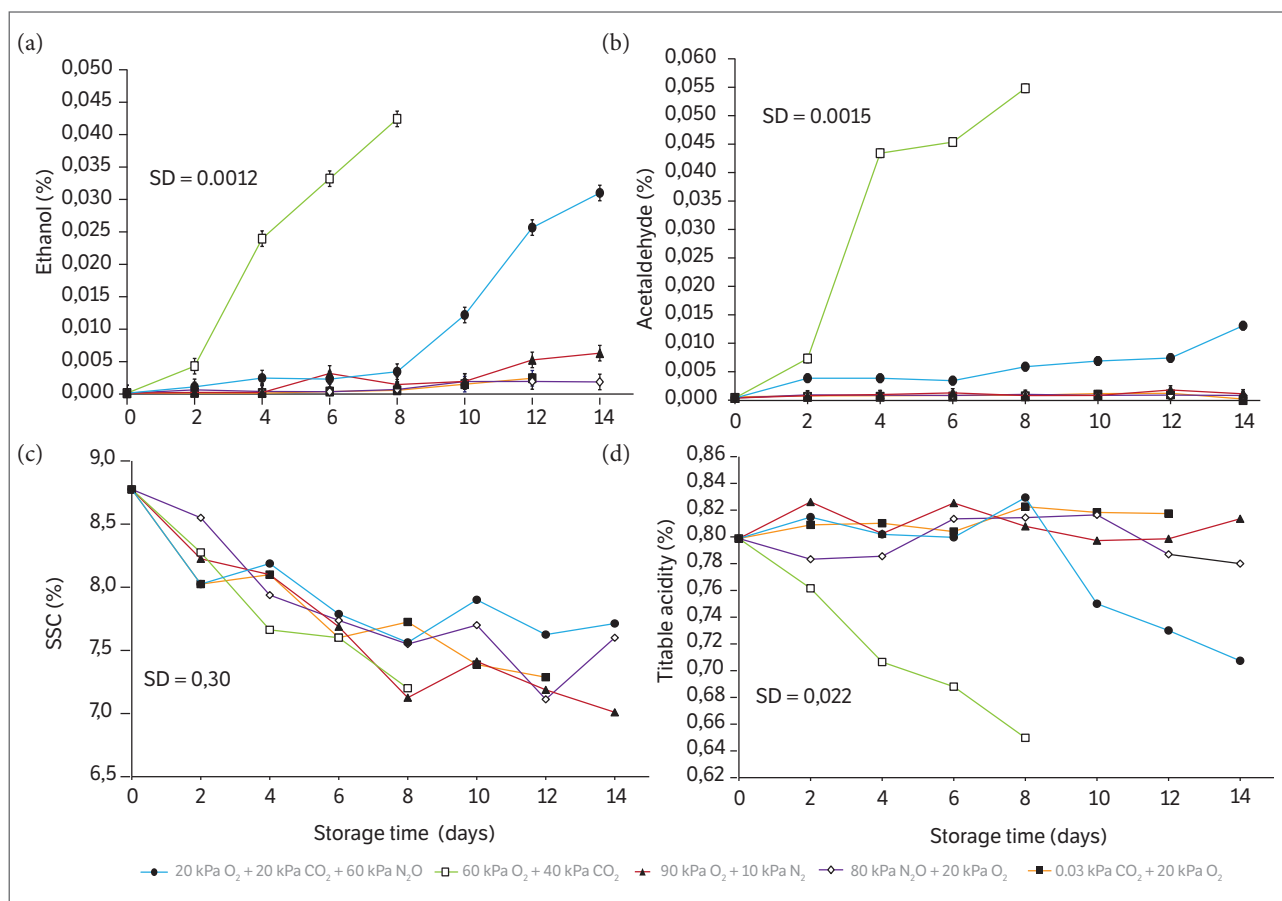
Strawberries under 20 kPa O<sub>2</sub> + 20 kPa CO<sub>2</sub> + 60 kPa N<sub>2</sub>O produced 0.0309% of ethanol and 0.0131% of acetaldehyde by day 14, without releasing any odors associated with fermentative processes. The other treatments did not result in significant changes to ethanol and acetaldehyde levels (Figs. 1a and b).

The different treatments had little effect on the physicochemical parameters. Only a reduction in SSC of 7% was observed in fruit stored at 90 kPa O<sub>2</sub> + 10 kPa N<sub>2</sub> at day 14 (Fig. 1c). Fruit kept at 60 kPa O<sub>2</sub> + 40 kPa CO<sub>2</sub> and 20 kPa O<sub>2</sub> + 20 kPa CO<sub>2</sub> + 60 kPa N<sub>2</sub>O underwent a reduction in TA (Fig. 1d), and a sharper reduction in firmness (Fig. 2a). Nevertheless, there was a tendency to reducing firmness in all treatments. Fruit stored at 0.03 kPa CO<sub>2</sub> + 20 kPa O<sub>2</sub>

**Table 1.** Incidence of postharvest decay (%) in 'Oso Grande' strawberries stored under different gas mixtures at 10 °C and 95% relative humidity.

Time (days)	Treatments (gas mixtures)				
	0.03 kPa CO <sub>2</sub> + 20 kPa O <sub>2</sub>	80 kPa N <sub>2</sub> O + 20 kPa O <sub>2</sub>	90 kPa O <sub>2</sub> + 10 kPa N <sub>2</sub>	60 kPa O <sub>2</sub> + 40 kPa CO <sub>2</sub>	20 kPa O <sub>2</sub> + 20 kPa CO <sub>2</sub> + 60 kPa N <sub>2</sub> O
0	(0.0*) 1.57Aa**	(0.0) 1.57Aa	(0.0) 1.57Aa	(0.0) 1.57Aa	(0.0) 1.57Aa
2	(2.7)1.41Bb	(0.0) 1.57Aa	(0.0) 1.57Aa	(0.0) 1.57Aa	(0.0) 1.57Aa
4	(7.4) 1.30Cd	(3.3) 1.39 Bc	(1.3) 1.46Bb	(0.0) 1.57Aa	(0.0) 1.57Aa
6	(13)1.21Dd	(5.3)1.34Cc	(5.3) 1.34Cc	(0.0) 1.57Aa	(1.3) 1.46Bb
8	(14) 1.19Ed	(7.3) 1.30Db	(8.0) 1.28Dc	(0.0) 1.57Aa	(1.5) 1.45Ca
10	(27) 1.02Fc	(10) 1.25Eb	(10) 1.25Eb	-	(2.5) 1.41Da
12	(52) 0.77Gd	(16) 1.16Fb	(18) 1.13Fc	-	(4.5) 1.36Ea
14	-	(19) 1.12Gb	(23) 1.07Gc	-	(5.7) 1.33Fa

\* Mean of 110 fruit expressed as a percentage. \*\* Transformed means ( $\arcsin \sqrt{x/100}$ ) when followed by a common letter, uppercase for the column or lowercase for the lines, do not differ from each other according to Tukey's test ( $p < 0.05$ ).



**Figure 1.** Changes to physicochemical variables in 'Oso Grande' strawberries stored under different gas mixtures, at 10° C and 95% relative humidity. (a) Ethanol levels (g of ethanol 100.g<sup>-1</sup> fresh plant material, %); (b) Acetaldehyde (g of acetaldehyde 100.g<sup>-1</sup> fresh plant material, %); (c) Soluble solid contents (%); and D) Titratable acidity (g of citric acid 100.g<sup>-1</sup> of pulp, %). Vertical bars in eachgraph represent the standard error from the mean.

and 90 kPa O<sub>2</sub> + 10 kPa N<sub>2</sub> were the only ones to change in L\*, becoming darker. Moreover, these strawberries went from light red to dark red as indicated by the change in hue angle (Figs. 2b and c).

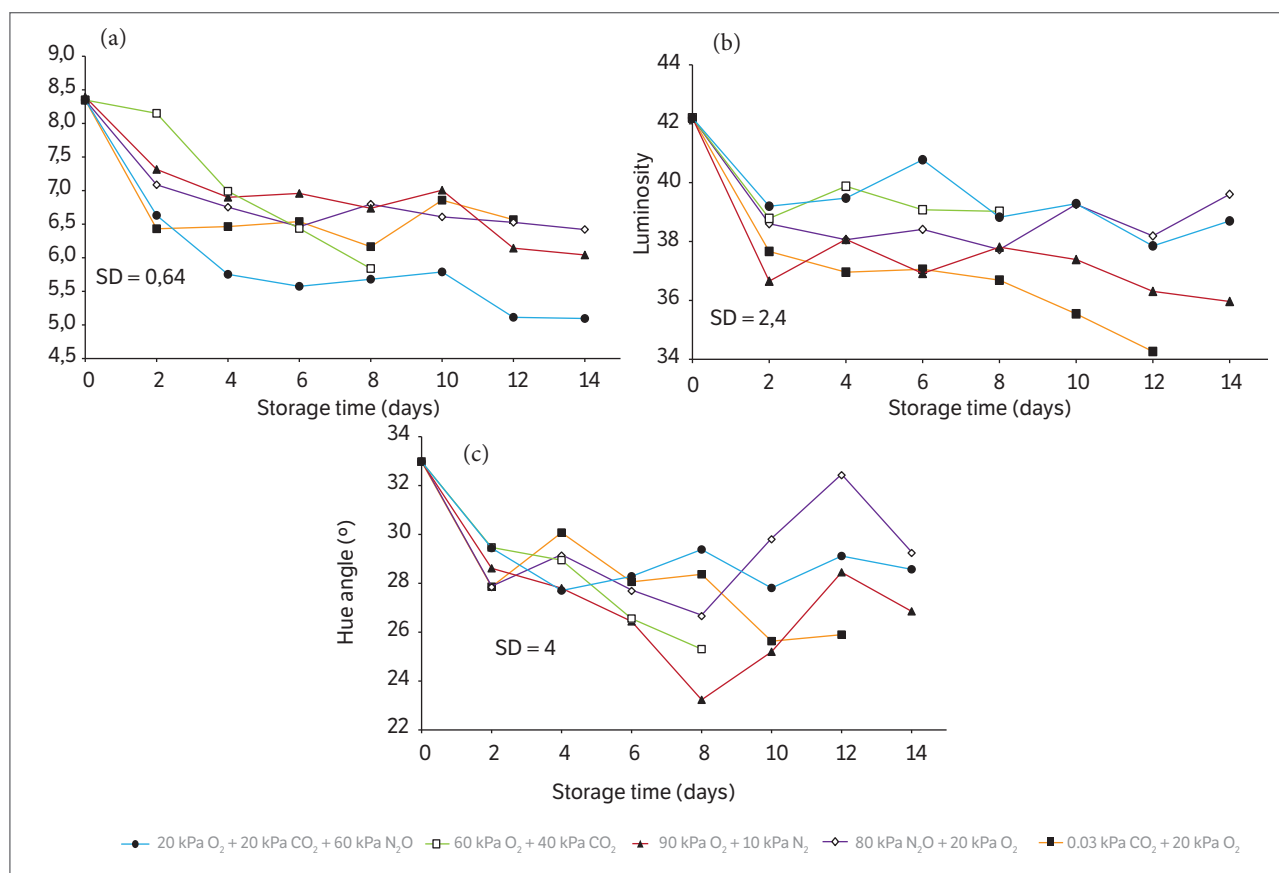
Results from the univariate analysis and from the comparison of standard errors of the mean allowed us to identify the isolated effect of the quality parameter during strawberry storage under different treatments. The highest fruit preservation benefits were observed under N<sub>2</sub>O atmosphere (80 kPa N<sub>2</sub>O + 20 kPa O<sub>2</sub>) and under high CO<sub>2</sub> concentrations (20 kPa O<sub>2</sub> + 20 kPa CO<sub>2</sub> + 60 kPa N<sub>2</sub>O). Also, by means of the O<sub>2</sub>-rich treatment (90 kPa O<sub>2</sub> + 10 kPa N<sub>2</sub>), which maintained strawberry storage time for 14 days at 10 °C (Table 1). These treatments reduced the incidence of postharvest decay, they maintained ethanol and acetaldehyde at a level that was similar to control (0.03 kPa CO<sub>2</sub> + 20 kPa O<sub>2</sub>) (Figs. 1a and b) and they delayed changes to SSC, TA, color and firmness (Figs. 1c, d and Fig. 2a).

These results may arise from the inhibitory effects of N<sub>2</sub>O and O<sub>2</sub> on the development of pathogens and/or from the activation of fruit defense mechanisms (Qadir and Hashimaga, 2001; Cunha Junior et al., 2013), but also from the synergistic association between CO<sub>2</sub> and N<sub>2</sub>O.

Isolated beneficial effects of CO<sub>2</sub> (Brackmann et al., 2001; Cunha Junior et al., 2012) and N<sub>2</sub>O (Qadir and Hashimaga, 2001; Cunha Junior et al., 2013) were previously described, but not in association with each other for the preservation of fresh fruit, specifically strawberries. The underlying reasons for the effects elicited by an O<sub>2</sub>-rich atmosphere have not been elucidated yet. Previous studies point to the potential activation of plant defense mechanisms (Zheng et al., 2008).

In this context, Zheng et al. (2008) reported low disease incidence under high O<sub>2</sub> concentrations, whereas Wszelaki and Mitcham (2000) considered this condition stressful to 'Camarosa' strawberries. These contrasting results may reflect

→



**Figure 2.** Changes to firmness (Newton, a), luminosity (b) and hue angle (c) in 'Oso Grande' strawberries stored under different gas mixtures, at 10 °C and 95% relative humidity. Vertical bars in each graph represent the standard error from the mean.

different gas mixtures or the distinct genetic background of cultivars, and warrant further studies.

## Multivariate analysis

### Factorial analysis

The univariate analysis clarified a number of issues, but it did not allow for the identification of relationships among quality parameters. In order to investigate these relationships, factorial analysis was applied with senescence as factor 1 and injury induced by AC as factor 2, which were related as distinct processes (Table 2).

The two first factors explained 65.7% of the total variance in the original data. Factor 1 (senescence) contributed with 34% of the variance in the original data and it presented a positive correlation to the variables SSC,  $L^*$ ,  $a^*$ , firmness and decay incidence. All of them represented quality parameters associated with strawberry senescence (Table 2). Factor 1 had a negative correlation with storage time. In other words, SSC,  $L^*$ ,  $a^*$  and firmness (Table 2) decreased with increasing

storage time under different CA conditions. It is important to note that postharvest decay increased with storage period. →

**Table 2.** Result of the factorial analysis conducted in 'Oso Grande' strawberries stored under different gas mixtures, at 10 °C and 95% relative humidity.

Variables	Factor 1	Factor 2
Soluble solids content	<b>0.86<sup>a</sup></b>	0.21
Ascorbic acid	0.37	0.43
Titrateable acidity	-0.08	<b>0.92</b>
Luminosity	<b>0.91</b>	-0.16
Hue	<b>0.87</b>	0.18
Chromaticity	-0.08	0.02
Firmness	<b>0.65</b>	0.23
Acetaldehyde	-0.03	<b>-0.92</b>
Ethanol	-0.12	<b>-0.95</b>
**Disease incidence	<b>0.72</b>	-0.36
Variance explained (%)	34.14	31.57
Interpretation	Senescence	CA-induced injury

<sup>a</sup> Factor coefficients in bold were used for interpretation, using index >0.60.

\*\* Transformed means (arcsine  $\sqrt{x/100}$ ).



However, since the data were transformed (Nishijima et al., 1992), higher incidence corresponds to lower arc-sine values (Table 1).

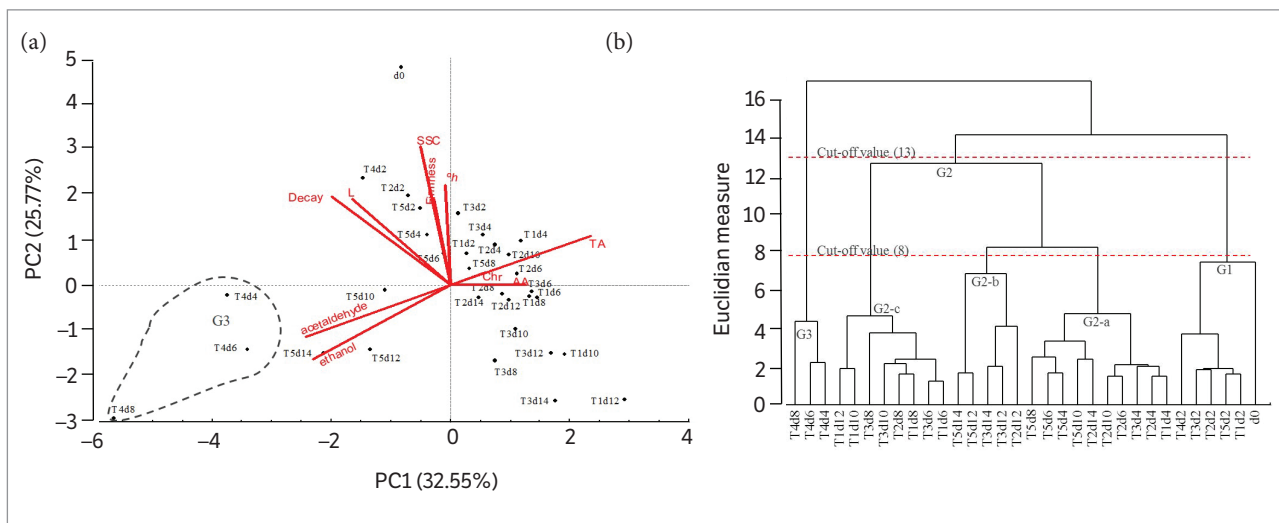
In support of these findings, Gil et al. (1997) and Cunha Junior et al. (2013) reported that strawberries stored under CA had reduced aromatic properties, flavor and brightness caused by deterioration or by the beginning of the rotting process. Pérez and Sanz (2001) as well as Wszelaki and Mitcham (2000) reported that strawberries stored in O<sub>2</sub>-rich conditions displayed reduced SSC. Wszelaki and Mitcham (2000) used 'Camarosa' strawberries kept under 40, 90 and 100 kPa of O<sub>2</sub> and they observed a decrease in the firmness of the fruit. Lastly, Gil et al. (1997) reported a reduction in hue in strawberries stored under CA (20 and 40 kPa CO<sub>2</sub>). All of these modifications are associated with fruit senescence.

Factor 2 (CA-induced injury) explained 31% of the variance in the original data. It also displayed a positive correlation with TA and a negative correlation with the levels of acetaldehyde and ethanol (Table 2). The observed injuries are closely related to the process of anaerobic respiration, which was triggered in strawberries by high CO<sub>2</sub> concentrations 60 kPa O<sub>2</sub> + 40 kPa CO<sub>2</sub> and 20 kPa O<sub>2</sub> + 20 kPa CO<sub>2</sub> + 60 kPa N<sub>2</sub>O. This process causes the acetaldehyde and ethanol production (Figs. 1a, b), and it explaining the higher levels these compounds in 60 kPa O<sub>2</sub> + 40 kPa CO<sub>2</sub> and 20 kPa O<sub>2</sub> + 20 kPa CO<sub>2</sub> + 60 kPa N<sub>2</sub>O in comparison to other treatments during throughout the storage period.

High CO<sub>2</sub> concentrations, above 20 kPa at different exposure times, may cause changes in normal fruit metabolism. They also reduce pyruvate dehydrogenase activity and induce pyruvate decarboxylase, alcohol dehydrogenase and lactate dehydrogenase, which elevate the production of acetaldehyde, ethanol, ethyl acetate, ethyl lactate. In addition, they may cause unwanted odors (Kader, 2003; Zhang and Watkins, 2005).

Fernández-Trujillo et al. (2007) attributed the increase in these compounds to anaerobic respiration in 'Jewel' strawberries stored under a CO<sub>2</sub>-rich atmosphere (20 kPa). Perez and Sanz (2001) also reported elevated production of acetaldehyde and ethanol in 'Camarosa' strawberries stored under an atmosphere containing 80 kPa O<sub>2</sub> + 20 kPa CO<sub>2</sub>.

Factor 2 was also associated with a reduction in TA (Fig 1d), especially for fruit subjected to treatments 20 kPa O<sub>2</sub> + 20 kPa CO<sub>2</sub> + 60 kPa N<sub>2</sub>O and 60 kPa O<sub>2</sub> + 40 kPa CO<sub>2</sub>. With this, they confirmed the positive correlation for this factor coefficient (Table 2). The reducing TA resulted from the increasing ethanol levels. Alcohol was produced to generate NAD<sup>+</sup> that feeded into the glycolytic pathway, but this process was not paralleled by the generation of H<sup>+</sup> and this has been proposed as a limiting process to cytoplasmic acidification (Blanch et al., 2015). Ethanol production uses H<sup>+</sup> and thus it increased pH and it reduced TA in the pulp of strawberries stored under high concentrations of CO<sub>2</sub> (Holcroft and Kader, 1999). The same pattern was observed by Gil et al. (1997) who reported a reduction in TA. This



**Figure 3.** Two-dimensional graph of the two first principal components (a) and dendrogram (b) showing the distribution of 'Oso Grande' strawberries that were stored under different gas mixtures at 10 °C and 95% relative humidity for 14 days. **T1**- 0.03 kPa CO<sub>2</sub> + 20 kPa O<sub>2</sub>, **T2**- 80 kPa N<sub>2</sub>O + 20 kPa O<sub>2</sub>, **T3**- 90 kPa O<sub>2</sub> + 10 kPa N<sub>2</sub>, **T4**- 60 kPa O<sub>2</sub> + 40 kPa CO<sub>2</sub> and **T5**- 20 kPa O<sub>2</sub> + 20 kPa CO<sub>2</sub> + 60 kPa N<sub>2</sub>O. **d0** – day zero before controlled atmosphere (CA), **d2** – 2nd day under CA, **d4** – 4th day under CA, **d6** – 6th day under CA, **d8** – 8th day under CA, **d10** – 10th day under CA, **d12** – 12th day under CA, and **d14** – 14th under CA.



happened when 'Selva' strawberries were stored under CA with 40 kPa CO<sub>2</sub>.

## PCA

PCA was used to visualize the distribution of the samples in a two-dimensional plan and to analyze the power of each parameter through the vectors formed (red lines, Fig. 3a). The two first principal components (PC1 and PC2) explained 58.3% of the variance in the original data (Fig. 3a).

When correlating quality parameters in PC1 and PC2 (biplot), it was observed that acetaldehyde and ethanol production contributed directly to the separation of fruit stored under 60 kPa O<sub>2</sub> + 40 kPa CO<sub>2</sub> for 4, 6 and 8 days. In addition, the fruit stored under 20 kPa O<sub>2</sub> + 20 kPa CO<sub>2</sub> + 60 kPa N<sub>2</sub>O for 12 days and 14 days from fruit in other treatments. The parameters chromaticity (Chr) and ascorbic acid (AA) had little effect on cluster formation.

PCA indicated that strawberry, stored under CO<sub>2</sub> rich conditions, tended to cluster in alignment with the vectors of ethanol and acetaldehyde production, and in opposition to the TA vector (Fig. 3a). Fruit under 20 kPa O<sub>2</sub> + 20 kPa CO<sub>2</sub> + 60 kPa N<sub>2</sub>O, which contained both CO<sub>2</sub> and N<sub>2</sub>O after day 10 at 10 °C, showed signs of anaerobic respiration as these strawberries clustered with those under 60 kPa O<sub>2</sub> + 40 kPa CO<sub>2</sub> (Fig. 3a).

Fruit were at their best quality immediately after harvest at day zero and they clustered on the superior left side of PC2. Thus, samples on the left of PC2 and above PC1 had better quality (Fig. 3a). It can also be observed strawberries stored under 90 kPa O<sub>2</sub> + 10 kPa N<sub>2</sub> for 8 to 14 days, and 0.03 kPa CO<sub>2</sub> + 20 kPa O<sub>2</sub> for 10 to 12 days displayed an indirect correlation with postharvest decay incidence (Fig. 3a).

## HCA

The HCA was used to identify the condition that best preserved the fruit in the qualitative state found at day 0. At the cut-off value of 13 in Euclidian distance, three large groups were formed (Fig. 3b). Group 1 (G1), with the best quality, was formed by the data set derived from days 0 and 2 for all treatments. Group 2 (G2), with intermediate quality, was formed by samples from all treatments and storage days, except fruits below 60 kPa O<sub>2</sub> + 40 kPa CO<sub>2</sub> stored at 4, 6, and 8 days. These fruits had worse quality (fermentation) and formed group 3 (G3).

Applying a more rigorous cut-off value of eight, five groups were formed, which G1 and G3 remained the same, and G2 was subdivided into G2-a, G2-b and G2-c (Fig. 3b). As deterioration increases from the right to left, from G1 to G3, groups closer to G1, specifically G2-a, consisted of better quality strawberries, that more closely resemble the fruit of day 0. G2-a included fruit below 80 kPa N<sub>2</sub>O + 20 kPa O<sub>2</sub> for 14 days (Fig. 3b).

Other treatments also effectively preserved strawberries including 90 kPa O<sub>2</sub> + 10 kPa N<sub>2</sub> and 20 kPa O<sub>2</sub> + 20 kPa CO<sub>2</sub> + 60 kPa N<sub>2</sub>O, which had fruit in good conditions at day 14 (Fig. 3b). However, strawberries stored under 20 kPa O<sub>2</sub> + 20 kPa CO<sub>2</sub> + 60 kPa N<sub>2</sub>O displayed signs of anaerobic respiration starting at day 10 (Figs. 1a, b).

## CONCLUSION

'Oso Grande' strawberries when stored at 10 °C under CA conditions of 80 kPa N<sub>2</sub>O + 20 kPa O<sub>2</sub>; 90 kPa de O<sub>2</sub> + 10 kPa N<sub>2</sub>O and 20 kPa O<sub>2</sub> + 20 kPa CO<sub>2</sub> + 60 kPa N<sub>2</sub>O had postharvest shelf life of 14 days at 10 °C, with reduced postharvest decay and a good quality level.

Treatment with 60 kPa O<sub>2</sub> + 40 kPa CO<sub>2</sub> caused injuries (Factor 2) characterized by increased ethanol and acetaldehyde production early in the storage period.

Treatments 80 kPa N<sub>2</sub>O + 20 kPa O<sub>2</sub> and 90 kPa de O<sub>2</sub> + 10 kPa N<sub>2</sub>O produced the best results, without changes to metabolism, and providing the best alternatives for 'Oso Grande' strawberry storage.

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