

Postharvest quality of 'Brookfield' apple field-treated with naphthalene acetic acid alone or combined with other growth regulators

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ABSTRACT: The Gala cultivars represent about 65% of the Brazilian apple production, however, it has a short harvest period, making necessary to use technologies that anticipate or delay fruit maturation. A widely used technology is the preharvest application of growth regulators. The objective of this investigation was to evaluate the effect of preharvest application of naphthalene acetic acid (NAA) combined with aminoethoxyvinylglycine (AVG) and 2-chloroethylphosphonic acid (Ethepon), on the quality of 'Brookfield' apple at harvest and after eight months controlled atmosphere (CA) (1.2 kPa O₂ and 2.0 kPa CO₂) storage followed by seven days of shelf life at 20 °C. The treatments were: [1] Control: application of water only; [2] NAA (40 g·ha⁻¹); [3] NAA (40 g·ha⁻¹) plus Ethepon (2.0 L·ha⁻¹ 24% of active ingredient), and [4] NAA (40 g·ha⁻¹) plus AVG (0.83 kg·ha⁻¹ 15% of active ingredient). At harvest, fruit treated with NAA presented a higher level of starch degradation, ethylene production and respiration rate. Fruit treated with NAA plus AVG maintained better quality after eight months under CA storage plus seven days of shelf life, due to higher healthy fruit amount and higher flesh firmness, but this combination reduced the red skin color index. Additionally, NAA plus Ethepon may be an alternative to maintain the quality of 'Brookfield' apple during storage in comparison to the application of NAA isolated.

Key words: aminoethoxyvinylglycine, controlled atmosphere, ethepon, fruit quality, *Malus domestica*.

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INTRODUCTION

'Brookfield' apple is a spontaneous 'Royal Gala' mutant, usually with a more intense red color covering the skin than the other mutants of this cultivar (Fioravanço et al. 2010), which improves its acceptability by Brazilian consumers and also due to the good organoleptic characteristics (Weber et al. 2017). However, the short harvest window needs the adoption of technologies that anticipate or delay fruit maturation, such as the use of growth regulators (Brighenti et al. 2017; Scolaro et al. 2015).

Naphthalene acetic acid (NAA) is a synthetic auxin that reduces the preharvest fruit drop (Yuan and Li 2008), although it may enhance fruit softening (Li and Yuan 2008). This is due to the increase in the expression of aminocyclopropane-1-carboxylic acid (ACC) synthase genes and, consequently, to higher ethylene production (Li and Yuan 2008; Unrath et al. 2009), decreasing the storage period of 'Brookfield' apple (Brackmann et al. 2014; Brackmann et al. 2015a). This growth regulator enhances genes expression that are related to the biosynthesis and perception of ethylene, and consequently the cell wall degrading enzymes (Li and Yuan 2008; Yuan and Li 2008). Results of Yuan and Carbaugh (2007) showed that the combination of NAA plus aminoethoxyvinylglycine (AVG) was more effective in delaying fruit drop, than only the application of



NAA and AVG isolated in 'Golden Supreme' and 'Golden Delicious' apples. Ozkan et al. (2016) concluded that application of NAA isolated was not as effective as isolated application of AVG in preventing preharvest drops of 'Red Chief' apple.

Ethephon is a compound that releases ethylene (Brackmann et al. 2014). Ethephon spraying makes possible to anticipate the harvest and increases red color of 'Gala' apple (Brighenti et al. 2017). Nevertheless, it decreases the storage time due to the faster flesh firmness loss (Steffens et al. 2005), and may cause excessive fruit drop in the orchard (Singh and Shafiq 2008).

Aminoethoxyvinylglycine is a well widespread growth regulator, applied in order to slow down the apple maturation and preharvest fruit drop (Arseneault and Cline 2016). This growth regulator inhibits the ACC synthase activity, decreasing or even inhibiting the ethylene biosynthesis (Romani et al. 1982), which induces fruit drop at preharvest time (Yildiz et al. 2012). In addition, AVG reduces the gene expression of cell wall degrading enzymes, such as the polygalacturonases and cellulases (Li et al. 2010).

Studies demonstrated that AVG delays maturation, allows to extend the harvest window and maintains better fruit quality during storage, delaying the loss of fruit firmness (Unrath et al. 2009). However, AVG also decreases the development of red color of 'Gala' apple skin (Scolaro et al. 2015), although this was not observed on the epidermis of 'Brookfield' apple treated with AVG (Brackmann et al. 2015a).

The effect of these plant growth regulators on fruit drop and quality at harvest were well evidenced by researches, however, it is important to know its effect, isolated or in combination, on the postharvest quality maintenance of the fruit. The storage system most widely used in Brazil for apple storage is the conventional controlled atmosphere (CA) (Weber et al. 2013), in which the O₂ partial pressure is reduced and the CO₂ is increased, thereby reducing the metabolism of the fruit (Mazzurana et al. 2016; Lumpkin et al. 2014; Lumpkin et al. 2015). According to Weber et al. (2013), it is possible to store apples for up to nine months in CA, however, Brackmann et al. (2014) claim that fruit losses may occur during the storage due the decay incidence, flesh breakdown and mealiness, which can be influenced by growth regulators applied in preharvest. According to Ozkan et al. (2012), NAA application in 'Braeburn' apple resulted in fruit with low soluble solids, lower titratable acidity and high starch index when compared to the treatment without this growth regulator. Steffens et al. (2005) found that the application of AVG alone or combined with Ethephon reduced flesh firmness loss, decay incidence and the occurrence of physiological disorders, but Ethephon alone increased the decay incidence of 'Gala' apple after storage in CA.

Therefore, this study aimed to assess the effect of NAA treatment alone and its combination with AVG and Ethephon on the quality of 'Brookfield' apple at harvest and the potential of quality preservation after eight months storage under CA.

MATERIAL AND METHODS

Experimental material and treatments establishment

This research comprises two steps. The first step of the research was conducted in a commercial orchard located in the county of Vacaria (Rio Grande do Sul – Brazil), in which the plant regulators were applied to the trees, as described below. The second step was carried out at the Postharvest Research Center at the Federal University of Santa Maria (NPP-UFSM), where the fruit that received the application of growth regulators were stored. 'Brookfield' apple, grafted on M9 rootstock, received the following field treatments: [1] Control: the application of water only; [2] NAA: 40 g·ha⁻¹ applied seven days before harvest; [3] NAA (40 g·ha⁻¹) plus Ethephon: 2.0 L·ha⁻¹ of Ethrel (Bayer CropScience, Germany) 24% of active ingredient, applied 10 days before harvest; [4] NAA (40 g·ha⁻¹) plus AVG: 0.83 kg·ha⁻¹ of Retain (Valent BioSciences, USA, 15% of active ingredient) applied 30 days before harvest. The volume sprayed was 1,000 L·ha⁻¹.

The experiment was conducted in a randomized block design in which each treatment consisted of four replications each with eight plants, and the fruit were randomly picked from a height of 1.5 m on both sides in the four central plants. One row, among each block, did not receive any application of growth regulators, to prevent the interference of one regulator over the other. After harvest, the fruit were transported to the Postharvest Laboratory of the NPP-UFSM. Following, the fruit were homogenized and those with mechanical damages and/or defects were excluded. Four experimental samples of 25 fruit each were prepared per treatment making 100 fruit. The fruit were then placed in experimental CA-rooms with

0.233 m³, which were sealed and placed inside a refrigerated room at 1.5 ± 0.1 °C throughout the entire storage period. The temperature was controlled by an electronic thermostat and monitored daily through mercury bulb thermometers (with resolution of 0.1 °C) inserted in the pulp of an apple that was placed inside the refrigerating room. The relative humidity inside the experimental CA rooms was monitored with psychrometers and kept at $94\% \pm 2.0\%$.

After, the CA rooms were sealed and the atmospheres were established, the O₂ partial pressure was achieved by flushing nitrogen (N₂), in order to reach 1.2 kPa of O₂. The CO₂ partial pressure of 2.0 kPa was obtained by the accumulation of CO₂ in the experimental room released by the fruit. During the eight months of storage, approximately four times a day, the partial pressure of gases inside the CA-rooms were measured and corrected, if necessary, with the aid of an automatic O₂ and CO₂ control system (Valis – Lajeado, Brazil) connected to a Siemens gas analyzer (Ultramat 23, Germany).

The adjustment of the O₂ was done by injecting air into the experimental room. The excess of CO₂ released by the fruit was automatically scrubbed using an absorber containing 100 kg hydrated lime.

Physicochemical analyses

After harvest, the following quality parameters were evaluated:

- a. Starch index: After cutting the fruit, a solution with 12 g of metallic iodine and 24 g of potassium iodide in 1 L of distilled water was applied to the peduncle half of the fruit. Afterwards, the reaction of iodine with fruit starch was compared with the photo table developed by Streif (1984), in which index 1 indicates fruit with maximum starch content, and index 10 represents fruit with fully hydrolyzed starch;
- b. Flesh firmness: Measured by a hand penetrometer (Effegi, model FT 327, 3-27 lbs., Milan, Italy) equipped with a 11 mm probe. Firmness was measured at the equatorial region of the fruit, both sides of the fruit were assessed after the removal of the skin. The results were expressed in Newton (N);
- c. Titratable acidity: Determined by titration of 10 mL juice in 100 mL distilled water with a 0.1 N solution of NaOH until it reaches pH 8.1. The results were expressed in meq 100 mL⁻¹;
- d. Soluble solids (SS): Determined by a hand refractometer (Biobrix, Model 103, Curitiba, Brazil), using three drops of juice extracted from each sample of 25 fruit, with a juice extractor (Philips Walita), which were placed on the refractometer prism to determine the soluble solids content. The results were expressed in °Brix;
- e. ACC oxidase activity: Measured according to the methodology developed by Bufler (1986). Therefore, 3 g of skin samples from equatorial region of 15 fruit were extracted per repetition and immediately immersed in a solution containing 0.1 mM·L⁻¹ ACC in 10 mM·L⁻¹ MES (2-(N-morpholino) ethanesulfonic acid) buffer at pH 6.0. After 30 min, the samples were placed in to hermetic 50 mL syringes together with 2% CO₂. After further 30 min, the ethylene concentration in the syringes was measured by gas chromatography (as described in the ethylene section). The data was expressed in $\mu\text{g C}_2\text{H}_4 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$;
- f. Ethylene production: Approximately 1,500 g of apple from each replicate were placed and sealed into 5 L glass jars for 1 h in air, at 20 °C. After the time incubation, two samples of 1 mL from the headspace of each glass were taken and injected into a Varian Star CX3400 gas chromatograph (Varian, Palo Alto, USA) equipped with a flame ionization detector (FID) and 2.0 m Porapak N80/100 steel column, for the ethylene production analysis. The temperature of the column, the injector and the detector were 90, 140 and 200 °C, respectively. The production of ethylene was expressed in $\mu\text{g C}_2\text{H}_4 \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$;
- g. Respiration rate: Expressed by the CO₂ release of fruit samples. It was measured using the same glasses and fruit samples of the ethylene production. The CO₂ released by the fruit was then measured with an electronic gas analyzer (model Oxycarb 6, Isolcell, Italy). The results were expressed in mg CO₂ kg⁻¹·h⁻¹. All the physicochemical and biochemical attributes described above, with exception of starch index, were evaluated after eight months storage followed by seven days of shelf-life in air, at 20 °C, as previously described. In addition, the following parameters were also evaluated at storage end:

- h. Internal ethylene concentration (IEC): Determined by withdrawing the air from the fruit using a vacuum pump (565 mm Hg during 2 min), according to Mannapperuma et al. (1991). The vacuum pump removed the air from a sample of fruit that were inside a container with water. An inverted funnel with a rubber septum at its thinnest end covered the fruit inside the container with water and allowed the air been removed from the interior of the fruit to accumulate within this funnel. Two samples of 1 mL of this air were drawn and injected into the injector port of the same chromatograph used for ethylene evaluation. The results were expressed in $\mu\text{g C}_2\text{H}_4\cdot\text{L}^{-1}$;
- i. Internal fruit space: A sample of approximately 50 g of pulp taken from 10 apples was submerged in distilled water and subjected to vacuum (565 mm Hg) for approximately 2 min until all the air in the intercellular spaces was filled by water. The intercellular space was obtained by the weight difference, before and after infiltration of the water in the sample. The results were expressed in percentage;
- j. Internal CO_2 concentration (ICO_2): Determined from two samples of the same air extracted for IEC, which were injected into the DANIGAS chromatograph (Dani Instruments Spa, Viale Brianza, Cologno Monzese, Italy), equipped with a Carboxen capillary column. The temperature of the injector, column and detector were 180, 90 and 230 °C, respectively. The results were expressed in $\text{mg de CO}_2\ 100\ \text{m}\cdot\text{L}^{-1}$.
- k. Gas diffusion: Assessed according to the method proposed by Anese et al. (2016). The results were expressed in $\text{mL CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$;
- l. Flesh breakdown: Evaluated in 25 fruit per repetition, totaling 100 fruit, which were cut in half. Then, the two halves were visually evaluated for the presence of pulp browning, characteristic of disorder. Values were expressed in percentages of affected fruit;
- m. Mealiness: All fruit of each replicate were cut in half in the equatorial region and any symptom of mealy pulp were evaluated. Fruit that presented a floury aspect (dry pulp and without juiciness) were classified as mealy pulp. The results were expressed in percentage of total fruit that presented symptoms of this disorder;
- n. Decay: All the apples that presented rottenness greater than 5 mm of diameter were considered. Data was expressed in percentage;
- o. Healthy fruit: All fruit that did not exhibit rot and internal or external disorders were considered healthy. Data was expressed in percentage;
- p. Red color index: Each fruit was individually evaluated in relation to the red color covering the skin, and classified according to a scale (0 to 3), in which 0 = up to 25% of red coverage; 1 = 26 to 50% of coverage; 2 = 51 to 75% of coverage; and 3 = 76 to 100% of red color. This index was obtained by multiplying the number of fruit classified in each index by the index number. Then, with the sum of these multiplications, the result was divided by the number of fruit evaluated in each sample;
- q. Fruit color: Determined with a Minolta colorimeter (model CR 310, Tokyo, Japan), which carries out measurement by the CIE L a*b* system, where L = luminosity (0 = black and 100 = white), a* indicates the variation from green (-a*) to red color (+a*) and b* is the variation of blue (-b*) to yellow color (+b*). The intensity of the color (chroma) and the hue angle of the red color were calculated from the values a* and b*;
- r. Mass loss: obtained from the weight difference of the fruit before and after storage. The results were expressed in percentage of mass loss.

Statistical analysis

A principal component analysis (PCA) was performed with all data, using the Unscrambler X software (version 9.7, CAMO A/S, Trondheim, Norway). Before carrying out the PCA, the data matrix was automatically sized for each variable to obtain the same weight for all variables (mean = 0 and variance = 1).

The data were submitted to analysis of variance (ANOVA), and the means were compared by the Tukey's test, at 5% probability of error. The parameters that did not present a normal distribution were transformed by the formula $\text{arc.sin } \sqrt{x/100}$, before the analysis of variance.

RESULTS AND DISCUSSION

Quality analysis at harvest

After the fruit were harvested, physical and chemical analyses were performed to characterize the maturity of the set of fruit from each treatment applied on the field (Table 1). In the present study, the fruit that received isolated NAA application did not differ statistically from the control. However, the combination of NAA with other growth regulator resulted in higher starch content than in fruit with isolated NAA and control treatments. According to Wang and Dilley (2001), the starch degradation occurs due to the induction of ripening caused by the increase in ethylene production. The flesh firmness and the titratable acidity did not differ among the treatments evaluated at the harvest.

Table 1. Starch index, flesh firmness, titratable acidity, soluble solids, ACC oxidase activity, ethylene production and respiration rate of 'Brookfield' apple at harvest.

Treatments	Starch index (1-10)	Flesh firmness (N)	Titratable acidity (meq 100mL ⁻¹)	Soluble solids (°Brix)
Control ^a	7.74 ab*	89.27 ns	4.90 ns	11.90 a
NAA ^b	8.58 a	87.03	4.50	11.85 a
NAA plus Ethephon ^c	6.86 bc	87.14	4.70	11.65 a
NAA plus AVG ^d	5.61 c	90.78	4.45	11.10 b
CV (%)	8.62	1.79	4.73	0.91
Treatments	ACC oxidase (µg C ₂ H ₄ .kg ⁻¹ .h ⁻¹)	Ethylene production (µg C ₂ H ₄ .kg ⁻¹ .h ⁻¹)	Respiratory rate (mg CO ₂ .kg ⁻¹ .h ⁻¹)	
Control ^a	92.5a	0.625 b	14.5 b	
NAA ^b	50.0 ab	1.36 a	18.4 a	
NAA plus Ethephon ^c	31.3 b	0.36 bc	11.7 bc	
NAA plus AVG ^d	93.8 a	0.12 c	11.2 c	
CV (%)	33.17	27.42	8.18	

^aControl: Only water application before fruit harvest; ^bNAA: Auxin application seven days before harvest; ^cEthephon: Ethrel application 10 days before harvest; ^dAVG: applied 30 days before harvest, by commercial product Retain; *Means followed by same letters in the columns do not differ by Tukey's test, at 5% probability (p < 0.05)

The higher starch content in the fruit treated with NAA plus AVG resulted in lower SS in relation to the other treatments (Table 1). According to Scolaro (2015), the application of AVG decreased the SS, as a result of delayed starch to sugar conversion. The SS are mainly composed by sugars. Probably, the inhibition of ethylene biosynthesis with AVG treatment was responsible for the lower starch degradation and lower SS content at harvest (Yuan and Carbaugh, 2007).

The fruit treated with NAA plus AVG did not differ from the control and those with application of isolated NAA with respect to ACC oxidase activity. However, the application of NAA plus Ethephon reduced the enzyme activity, in comparison to the samples treated with NAA plus AVG (Table 1). This increase of the ACC oxidase enzyme activity in the fruit treated with NAA plus AVG did not result in higher ethylene production and respiration rate, probably due to effect of AVG blockage on the production of ACC by the inhibition of the ACC synthase. This enzyme is inhibited by AVG application and is responsible for the production of the ethylene precursor ACC (Ju and Curry 2000). Previous studies have shown that the application of NAA increased the expression of ACC synthase genes, consequently increasing the production of ethylene (Yu and Yang 1979; Li and Yuan 2008). At the present study, the application of isolated NAA did not increase the ACC oxidase activity compared to the control, in the evaluation made at harvest.

The highest ethylene production and respiration rates were found in NAA-treated fruit (Table 1). The preharvest application of NAA decreases fruit abscission due to the reduction in gene expression of enzymes involved in the degradation of cell wall in the abscission zone (Li and Yuan 2008), since the application of NAA increases auxin levels in fruit (Yuan and Carbaugh 2007). There is a complex relationship among auxin and ethylene functions in apple fruit, which are related to gene family members and fruit developmental stages (Shin et al. 2015). The NAA increases the expression of genes related to the ethylene biosynthesis (*MdACS1* and *MdACO1*), perception (*MdERS1*) and cell wall degradation enzymes (*MdPG1*)

(Li and Yuan 2008; Yuan and Li 2008), therefore decreasing the storage potential of the fruit (Brackmann et al. 2014; Brackmann et al. 2015a). The high respiration rate measured in this treatment is probably related to the higher ethylene production observed in NAA-treated fruit, since respiration rate has a close relation with the ethylene production (Both et al. 2016).

The treatment with NAA plus AVG inhibited the production of ethylene and reduced the respiration rate of these fruit (Table 1). Similar results were obtained in ‘Delicious’ apple, where an inhibition of ethylene production in the fruit treated with NAA plus AVG was detected (Li and Yuan 2008). The lower production of ethylene may be related to the inhibition of ACC synthase enzyme activity by AVG (Ju and Curry, 2000), which triggers the conversion of S-adenosyl-methionine (SAM) to ACC, the precursor of ethylene (Yu and Yang 1979).

Quality analysis after eight months of storage

An analysis of the principal components was performed to show an overview of the variables analyzed and the effect of the treatments (Fig. 1a,b). The PC I and PC II axes explain 61.33 and 27.15%, respectively, that is, 88.48% of the total variation of the parameters evaluated. Figure 1a corresponds to the treatments and Fig. 1b to the variables. The PC I separates the fruit treated with NAA plus AVG from all the other treatments, whilst PC II discriminates the fruit treated with NAA from the ones submitted to NAA plus Ethephon and Control (Fig. 1a). This result demonstrates that the combination of NAA plus AVG presents a response that is completely different from the other treatments, regarding the quality maintenance during the postharvest period, in accordance to the results verified by Brackmann et al. (2015a).

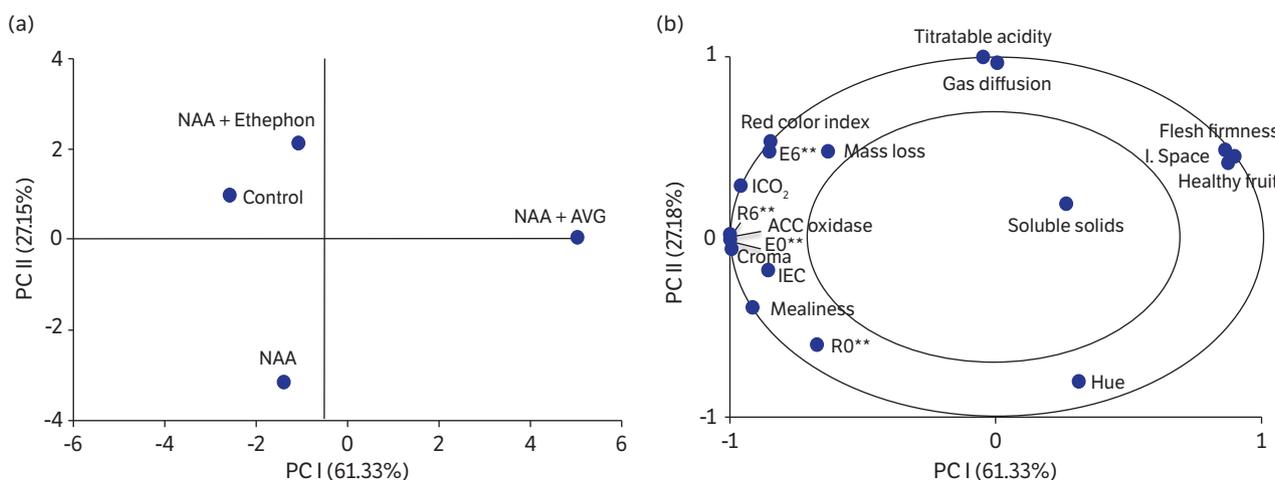


Figure 1. (a) Scores (treatments) and (b) loadings (variables) of the two major components of ‘Brookfield’ apple after eight months of controlled atmosphere storage plus seven days of shelf life at 20 °C. *Control: Only water application before fruit harvest. NAA: Auxins application seven days before harvest. Ethephon: Ethrel application 10 days before harvest. AVG: Applied 30 days before harvest, by the commercial product Retain. ** E0 and E6: Ethylene production at zero and six days of shelf life. R0 and R6: Respiratory rate at 0 and 6 days of shelf life.

The fruit treated with NAA plus Ethephon were comparable to the control treatment, because both treatments were located in the same quadrant of the PCA (upper left side) and presented correlation with the variables, gas diffusion, ethylene production (six days of shelf life), acidity, mass loss and internal CO₂ concentration. Moreover, a high red color index of the skin was observed with this treatment (Fig. 1b). The preharvest NAA application correlated with the respiration (right soon after room opening), mealiness, internal ethylene concentration and ACC oxidase activity (Fig. 1b). On the other hand, preharvest fruit treated with NAA plus AVG showed higher flesh firmness, internal space and greater percentage of healthy fruit after the storage period (Fig. 1a, b). These results are in agreement with previous studies, which concluded that the application of AVG maintains the quality in some apple cultivars (Steffens et al. 2006; Wang and Dilley 2001; Yuan and Carbaugh 2007; Yuan and Li 2008). The PCA also clearly showed that the application of AVG inhibited the damaging effect of NAA in the quality maintenance of ‘Brookfield’ apple after storage. Thus, this may be a recommendation to be

adopted in practice, since it is possible to control the preharvest drop without harm the storage potential, in addition to extending the harvest window.

After eight months of storage, the fruit in the control and those treated with NAA plus Ethephon showed elevated ethylene production during shelf life (Fig. 2a). This increased ethylene production probably resulted in a higher respiration rate (Fig. 2b) and higher IEC (Fig. 3b) in the fruit, which is confirmed by PCA, where both variables are in the same quadrant (Fig. 1b).

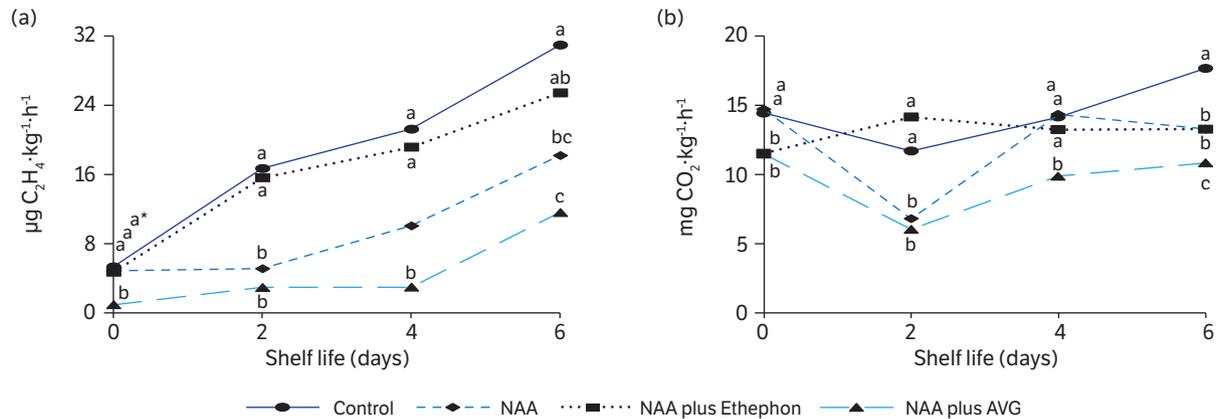


Figure 2. Ethylene production (a) and respiration rate (b) of 'Brookfield' apple after storage for eight months under controlled atmosphere (1.2 kPa O₂ plus 2.0 kPa CO₂) plus 0, 2, 4 and 6 days of shelf life at 20 °C. *Means followed by same letters on the same day do not differ by Tukey's test, at 5% probability ($p < 0.05$). ** Control: Only water application before fruit harvest. NAA: Auxins application seven days before harvest. Ethephon: Ethrel application 10 days before harvest. AVG: applied 30 days before harvest, by the commercial product Retain.

The lower ethylene production measured in fruit treated with the NAA plus AVG combination may be related to the lower ACC oxidase activity during shelf life (Fig. 3a). Previous studies have demonstrated that the application of AVG, an inhibitor of ethylene synthesis, appears to be an alternative to replace the isolated application of NAA, because it blocks the production of ACC by inhibiting ACC synthase and thus suppressing the production of ethylene (Yuan and Carbaugh 2007; Li and Yuan 2008). The combination of NAA plus AVG was also efficient in blocking ethylene biosynthesis in 'Golden Delicious' apples (Yuan and Carbaugh, 2007). The same authors also observed that the combined application of NAA plus AVG provided a lower ethylene production than the isolated application of NAA or AVG.

The fruit from plants treated with isolated NAA presented an intermediate ethylene production and respiration rate at six days of shelf life, compared to the other treatments (Fig. 2a, b). A previous study has reported that the isolated application of NAA increases the activity of the ACC oxidase enzyme and, thus intensifies the production of ethylene and respiration rate and, consequently, fruit ripening (Yuan and Carbaugh 2007). However, in this study, the fruit treated with NAA presented lower ethylene production when compared to the control (Fig. 2a). These fruit were probably already in an advanced maturation stage, that is, besides the climacteric peak, which can be evidenced by the greater production of ethylene and respiration at harvest (Table 1).

The fruit from the plants treated with growth regulators showed distinct responses throughout shelf life in relation to the respiration rate. Right after being removed from the chamber, the control samples and the ones treated with isolated NAA exhibited a higher respiration rate in comparison to the fruit from all the other treatments (Fig. 2b). Nevertheless, after two days of shelf life, the lowest respiration rate was observed in the fruit submitted to the application of NAA and NAA plus AVG. However, at six days, the lowest respiration rate was observed only in the fruit treated with NAA plus AVG, possibly due to the lower ethylene production and IEC (Fig. 2a and 3b). Although, the fruit in the control displayed the highest respiration rate of all treatments, it is important to note that the respiration rate has a close relation to ethylene production (Both et al. 2016) and may change according to the cultivar, mass loss, storage temperature and atmosphere in which the fruit are exposed (Pinto et al. 2012).

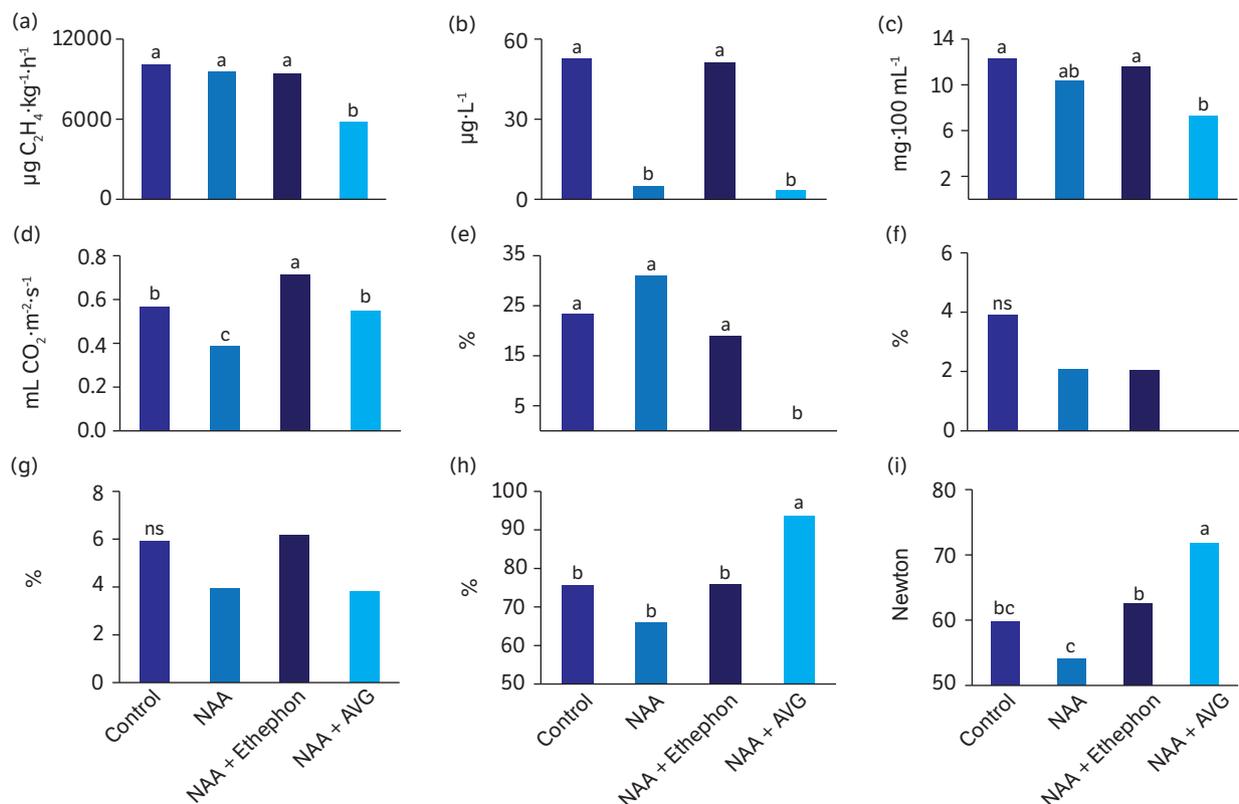


Figure 3. ACC oxidase activity (a), internal ethylene concentration (b), internal CO₂ concentration (c), gas diffusion (d), mealiness (e), flesh breakdown (f), decay (g), healthy fruit (h) and flesh firmness (i) of 'Brookfield' apple after storage for eight months in controlled atmosphere (1.2 kPa O₂ plus 2.0 kPa CO₂) plus seven days shelf at 20 °C. * Means followed by same letters do not differ by Tukey's test, at 5% probability (p < 0.05). ns: not significant difference. ** Control: Only water application before fruit harvest. NAA: Auxins application seven days before harvest. Ethephon: Ethrel application 10 days before harvest. AVG: applied 30 days before harvest, by the commercial product Retain.

In the present study, fruit from the control treatment, NAA and NAA plus Ethephon presented the highest ACC oxidase enzyme activity. NAA is involved in the expression of genes linked to the ACC oxidase enzyme activity, as the gene *MdACO1* (Li and Yuan 2008). On the other hand, fruit treated with NAA plus AVG presented lower activity of this enzyme (Fig. 3a). Possibly, the application of AVG before NAA reduced the activity of ACC oxidase by suppressing the genes involved in the expression of the ACC synthase enzyme (*MdACS1*) (Yuan and Carbaugh 2007; Li and Yuan 2008), required for the ACC production, which is the substrate of ACC oxidase enzyme (Yang and Hoffmann 1984).

The highest internal ethylene concentration (IEC) was observed in the fruit of the control treatment followed by those treated with NAA plus Ethephon (Fig. 3b). The higher IEC in the fruit from these treatments are related to the elevated ACC oxidase activity and ethylene production (Fig. 2a and 3a). Ethephon is a product that releases ethylene, stimulating the autocatalytic ethylene production, which likely promoted the highest IEC in the fruit. According to Steffens et al. (2006), fruit treated with Ethephon in preharvest presented higher ethylene production than fruit without Ethephon application. IEC was also elevated in 'Fuji' apple treated with Ethephon (Li et al. 2002). Furthermore, Brackmann et al. (2014) observed similar results while assessing IEC in 'Brookfield' apples treated with NAA plus Ethephon.

High internal concentration of CO₂ (ICO₂), can cause physiological disorders, especially in apples susceptible to high CO₂ concentrations (Castro et al. 2008). The fruit treated with NAA plus AVG showed the lowest ICO₂, differing statistically from the fruit treated with NAA plus Ethephon and the control (Fig. 3c).

Gas diffusion is an important evaluation for the fruit metabolism, seeing that the uptake of O₂ and release of CO₂ occur during fruit respiration, which need to be diffused by the fruit pulp (Ho et al. 2013). Very high concentration of CO₂ and low O₂ cause physiological disorders (Herremans et al. 2013; Ho et al 2013). In this study, the fruit treated with NAA isolated presented lower gas diffusion rate (Fig. 3d), suggesting an advanced fruit ripening, with lower cellular integrity.

This result is consistent with Brackmann et al. (2014), who found that the application of NAA isolated, resulted in a lower gas diffusion in the pulp of 'Brookfield' apple. The fruit treated with NAA plus Ethephon presented a higher gas diffusion rate. Fruit of the control and NAA plus AVG treatments had an intermediate gas diffusion, without significant difference among them (Fig. 3d).

Mealiness is one important physiological disorder for apples of the 'Gala' group, which is described by a farinaceous texture and loss of juiciness due to the middle lamella degradation and subsequent cell separation (Prasanna et al. 2007; Payasi et al. 2009), decreasing apple quality and possibly making it unsuited for commercialization (Weber et al. 2015). In this research, fruit of the control, NAA and NAA plus Ethephon treatments showed the highest mealiness occurrence (Fig. 3e). However, when the NAA was applied in combination with AVG, there was no mealiness in the fruit, which results in a higher percentage of healthy fruit in relation to the other treatments (Fig. 3h). This result is in agreement with Brackmann et al. (2015b), who observed higher percentage (around 90-95%) of healthy fruit treated with AVG alone or in combination with NAA, ethylene absorption (ABS) and 1-methylcyclopropene (1-MCP) application. The latter acts as an inhibitor of ethylene action in fruit cells (Yuan and Carbaugh 2007). This finding may be related to the lower ethylene production, lower IEC and lower ICO_2 (Figs. 2a, 3b,c). Ethylene is a key regulator for gene expression and activity of enzymes that degrade the cell wall (Payasi et al. 2009; Prasanna et al. 2007). A previous study also found a lower incidence of mealiness in the AVG plus NAA treatment in comparison to AVG isolated or in combination with Ethephon (Brackmann et al. 2015a).

Regarding to flesh breakdown (Fig. 3f), there was no statistical difference among the treatments. Previous researchers have found that a mass loss of 4% in 'Royal Gala' apple (Pinto et al. 2012) and of 3.5% in 'Maxi Gala' apple (Weber et al. 2013) during storage reduced the flesh breakdown occurrence. In this study, the lowest mass loss was found in the fruit treated with NAA plus AVG, not differing from the control fruit and the samples treated only with NAA, but the fruit treated with NAA plus Ethephon showed higher mass loss (Fig. 4c). Despite the significant difference among treatments concerning mass loss, this range was very close to the ideal mass loss of about 3% proposed by Anese et al. (2016), not affecting the flesh breakdown occurrence.

During long-term storage period, decay incidence is one of the main causes of apple fruit loss (Corrent et al. 2009; Vilanova et al. 2012; Vilanova et al. 2014). However, in the present study, no significant difference among treatments was measured (Fig. 3g). Nevertheless, there are studies that have found a lower rot incidence in 'Gala' apple when the fruit were treated with isolated application of AVG or in combination with Ethephon (Steffens et al. 2005). The fruit treated with NAA plus AVG showed the highest percentage of healthy fruit compared to all the other treatments (control, NAA and NAA plus Ethephon) (Fig. 3h), which is mainly related to the absence of mealiness (Fig. 3e). Brackmann et al. (2015a) found higher percentage of healthy fruit in the AVG and AVG plus NAA treatment in comparison to the control and AVG plus Ethephon.

The maintenance of flesh firmness after long-term apple storage is a desirable feature, since it is one of the main quality parameters considered by consumers (Brackmann et al. 2009; Harker et al. 2008). NAA plus AVG treatment maintained higher flesh firmness, compared to the fruit of the other treatments (Fig. 3i). The highest flesh firmness observed in this treatment is related to low ethylene production, respiration rate, ACC oxidase activity and mealiness (Figs. 2a,b, 3a, 3e). According to Payasi et al. (2009), the reduction in flesh firmness is related to cell wall degradation, triggered by enzymes, such as polygalacturonases and pectinamethylesterase, which have their activities increased by the ethylene action. However, application of NAA plus Ethephon outcome in fruit with higher flesh firmness compared to those treated only with NAA. The control treatment did not differ from these treatments (Fig. 3i), however, a benefit was obtained by applying NAA plus Ethephon in the preharvest by standardizing fruit maturation (Brackmann et al. 2014). Ozkan et al. (2016) verified less flesh firmness of 'Red Chief' apple in treatment with application of $20 \text{ mg}\cdot\text{L}^{-1}$ of NAA compared with different AVG concentrations, in the two evaluated years, which is in agreement with the results of this study.

Soluble solids and acidity are important characteristics for consumer preference (Harker et al. 2008). The titratable acidity was lower in fruit with application of NAA isolated and higher in the fruit treated with NAA plus Ethephon (Fig. 4a), but these presented lower soluble solids content compared to all the other treatments (Fig. 4b). The cell wall components solubilization, such as pectins, which are mainly composed by galacturonic acid, may increasing the juice acidity (Prasanna et al. 2007). This could help to explain the high acidity of the fruit treated with NAA plus ethephon, which showed high

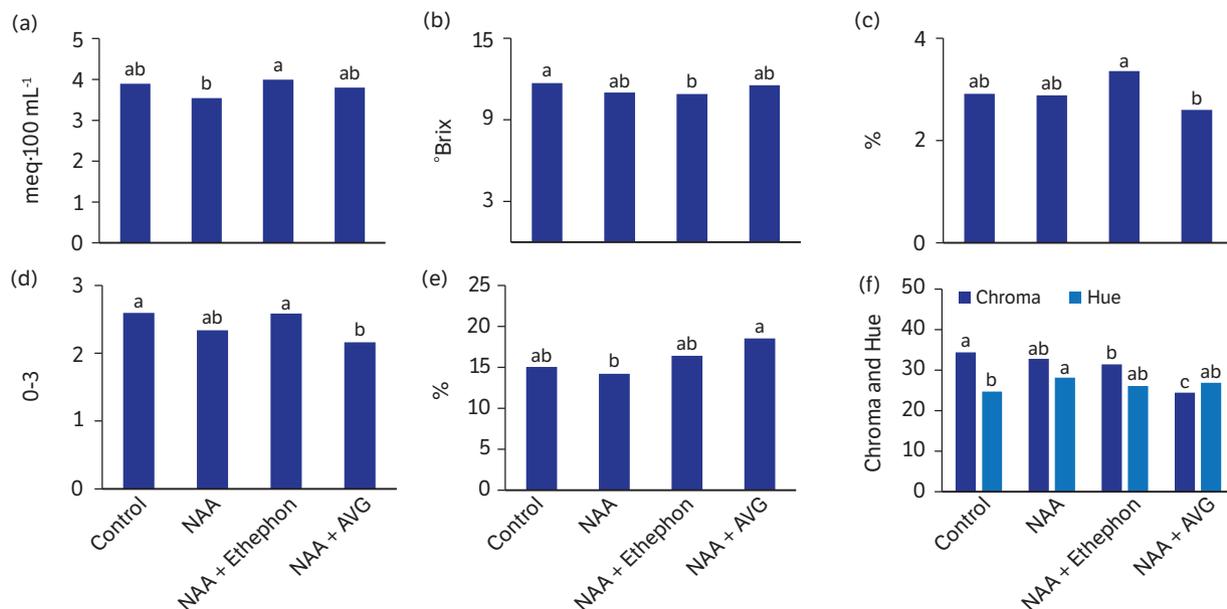


Figure 4. Titratable acidity (a), soluble solids (b), mass loss (c), red skin color index(d), internal space (e), and red color intensity of the skin (f) of 'Brookfield' apple after eight months storage in controlled atmosphere (1.2 kPa O₂ plus 2.0 kPa CO₂) plus seven days of shelf life at 20 °C. *Means followed by same letters do not differ by Tukey's test, at 5% probability ($p < 0.05$). ** Control: Only water application before fruit harvest. NAA: Auxins application seven days before harvest. Ethephon: Ethrel application 10 days before harvest. AVG: applied 30 days before harvest, by the commercial product Retain. *** Red skin color: evaluated subjectively by identifying the area of the epidermis with red color, according to a scale from 0 to 3: 0 = up to 25% of the red fruit skin; 1 = 26 to 50% of the red fruit skin; 2 = 51 to 75% of the red fruit skin; 3 = 76 to 100% of red fruit skin.

occurrence of mealiness and low flesh firmness, a result of cell wall solubilization. However, NAA isolated showed the lower flesh firmness (Fig. 3i), but also low acidity. In this case, these acids may be consumed by the fruit metabolism, result of the advanced maturity at harvest (higher starch index, ethylene production and respiration rate) and ripening after storage.

The fruit treated with NAA plus AVG showed a higher internal space compared to those treated with NAA (Fig. 4e), which may be related to the absence of mealiness and flesh breakdown, due to the lower metabolism (ethylene production and respiration rate), thus providing a higher amount of healthy fruit. Herremans et al. (2013) found that the smaller intercellular space causes a low diffusion of gases in the fruit tissue, which is in agreement with the results of this study.

The red color index was higher in fruit treated with NAA plus Ethephon and in the control, without differing significantly from the fruit treated with NAA isolated. On the other hand, NAA plus AVG treatment resulted in the lowest red color index (Fig. 4d). This demonstrates that AVG had a negative effect on this characteristic. Other studies have also reported that AVG application delayed the synthesis of anthocyanins in the epidermis of 'Pink Lady' apples (Whale et al. 2008) and 'Imperial Gala' (Petri et al. 2010). AVG inhibit ethylene synthesis, which resulted in lower red skin coloration in 'Gala' apples (Amarante et al. 2010; Scolaro et al. 2015). However, Brackmann et al. (2015a) did not found differences in the epidermis red skin color of 'Brookfield' apple when fruit were treated with AVG or combined with NAA or Ethephon.

Regarding the intensity of the background color (Chroma), fruit treated with NAA plus AVG showed the lowest Chroma value in comparison to the other treatments (Fig. 4f). The highest Chroma value was verified in the fruit of the control treatment with significant difference to those treated with NAA plus Ethephon and NAA plus AVG. In the evaluation of the red color hue angle, fruit treated with only NAA presented a higher hue angle, compared to the fruit of the control treatment, that is, the fruit in the control had a higher intensity of red color, being in agreement with the result verified for the red color index (Fig. 4d). Apple red skin coloration is mainly conferred by the amount and types of anthocyanins present (Awad and Jager 2002). According to Faragher and Brohier (1984), ethylene may be involved in the activity of the phenylalanine ammonia-lyase enzyme (PAL), which participates in the synthesis of anthocyanins, conferring a red color to the fruit.

CONCLUSION

The treatment of 'Brookfield' apple with NAA plus AVG resulted in better fruit quality after eight months of storage and shelf life, but delayed the development of the red skin color.

Ethephon followed by NAA application may be an alternative to maintain the quality of 'Brookfield' apple during storage in comparison to the application of NAA isolated, besides did not affect the red skin color.

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