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Quality of 'Baigent' apples as a function of pre-harvest application of aminoethoxyvinylglycine and ethephon stored in controlled atmosphere

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ABSTRACT

– The objective of this work was to evaluate the effects of pre-harvest application on 'Baigent' apple trees cultivated under black anti-hail nets with aminoethoxyvinylglycine (AVG), in a single or split dose, combined or not with ethephon, on the quality, the antioxidant activity, and the content of phenolic compounds of the fruits after storage in controlled atmosphere (CA). The pre-harvest applications of AVG alone, both at a dose of 0.125 g L^{-1}, as well as in split dose and at a dose of 0.0625 g L^{-1}, reduced the rate of ethylene production, showed less yellowing fruits and a lower incidence of cracks, maintaining high flesh firmness, texture attributes, and fruit acidity, without causing negative effects on the incidence of mealiness and decay. The pre-harvest applications of AVG at a dose of 0.125 g L^{-1} and in split dose combined with ethephon also contributed to the reduction of the ethylene production rate and yellowing, showed high flesh firmness and texture, however, it showed a high incidence of decay and cracking when ethephon was combined with AVG at a dose of 0.125 g L^{-1}. In the skin, application of AVG only at a dose of 0.125 g L^{-1} reduced the total phenolic compounds values, however, total antioxidant activity was reduced with any form of AVG application. In the flesh, there was no effect of pre-harvest application of AVG. Regardless of the dose and form of application, AVG reduced the contents of chlorogenic acid, phloridizin, and epicatechin in the skin of fruits harvested at commercial harvest.

1. Introduction

Apples (*Malus domestica* Borkh) are considered an excellent source of natural antioxidants, as they are composed of vitamin C and phenolic compounds (Bohn & Bouayed, 2020). These compounds are beneficial to human health due to their antioxidant potential (Ho et al., 2020; Mignard, 2021) and may prevent several chronic diseases (Starowicz et al., 2020).

Phenolic compounds are the substances that contribute the most to the antioxidant activity in apples (Li et al., 2021). They derive from secondary metabolism and perform essential functions in the fruit cell biochemistry, reproduction, growth, defense mechanisms, color, and flavor (Isah, 2019). Their antioxidant action is attributed to their molecular structure, in particular, the number and positions of hydroxyl groups, and the substitutions of aromatic rings, and to their ability to eliminate free radicals through the donation of hydrogen atoms (Minatel et al., 2017).

The main groups of phenolic compounds in apples are phenolic acids, dihydrochalcones, flavonoids, flavan-3-ols, and anthocyanins (Starowicz et al., 2020; Stanger et al., 2018). Phenolic acids, dihydrochalcones, and flavonoids contribute relatively little to the content of total phenolic compounds (TPC) in apples, with values between 3% and 30%, 1% and 5%, and 2% and 10%, respectively. On the other hand, flavan-3-ols, in monomeric [(+) - catechin and (-) - epicatechin] and oligomeric (proanthocyanidins) forms, contribute from 55% to 85% to the content of TPC in apples. Anthocyanins are present in red or partially red apple cultivars, as is the case of the variety Baigent, and represent between 1% and 7% of the TPC content (Ceymann et al., 2012).

The pre-harvest application of growth regulators can change the profile of phenolic compounds and the amount of TPC (Ozturk et al., 2013), as well as the total antioxidant activity (TAA) and the quality

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of the fruit (Soethe et al., 2019). Aminoethoxyvinylglycine (AVG) is a plant growth regulator applied to the fruit to delay the harvest, reducing the fall of fruit, and maintaining the quality during storage, as it also delays the loss of firmness of the fruit (Brackmann et al., 2014). AVG suppresses ethylene synthesis by inhibiting the activity of the 1-aminocyclopropane-1-carboxylate (ACC) synthase enzyme (Nascimento et al., 2018).

However, the application of AVG reduces the development of the red color in the skin of apples (Soethe et al., 2021), reducing its visual quality. This effect can be even more significant in cultures under anti-hail nets due to the reduction in light, which compromises the synthesis of anthocyanins (Mupambi et al., 2018). Nevertheless, in order to avoid the negative effect of AVG on the red color formation of the fruit skin, the application of ethephon (2-chloroethylphosphonic acid) to apple trees previously treated with AVG or the partial application of AVG may be viable alternatives (Soethe et al., 2019; 2021).

One of the most used storage systems for maintaining the quality of apples is the controlled atmosphere (CA), which allows for fruit storage for a period of up to nine months (Weber et al., 2013). In this storage system, fruit metabolism is reduced by decreasing the partial pressure of O_2 and increasing CO_2 inside the storage chambers (Mazzurana et al., 2016). According to Brackmann et al. (2014), the delay in the loss of fruit quality during storage can be influenced by the growth regulators used in pre-harvest.

Most of the research on AVG evaluates its application in a single-dose, 30 d before the commercial harvest. It is possible, however, that the partial application of AVG or the application of a reduced dose closer to the harvest, in addition to minimizing the effects of AVG on the red color of the fruit (Soethe et al., 2021), may still help to delay ripening and contribute to better fruit quality after storage. Moreover, no information was found on the effect of AVG on the control of the ripening of 'Baigent' apples produced under anti-hail nets, as well as on the functional properties of the fruit stored in CA. Anti-hail nets, due to their altering of the growing environment, can compromise the fruit quality, reducing the firmness of the flesh and the content of soluble solids, and delaying the development of the red color of 'Gala' apples (Amarante, Steffens, & Argenta, 2012), and interfere with the effect of AVG on the control of fruit ripening.

The objective of this work was to evaluate the effects of pre-harvest application on 'Baigent' apple trees cultivated under black anti-hail nets with AVG, in a single or split dose, combined or not with ethephon, on the quality, the antioxidant activity, and the content of phenolic compounds of the fruits after storage in CA.

2. Materials and methods

2.1. Orchard location

The experiment was carried out in the 2015/2016 season, in a commercial 'Baigent' apple (*Malus domestica*) orchard located in the city of Vacaria (Rio Grande do Sul, Brazil – 50° 42' W; 28° 33' S; 955 m altitude) covered with black anti-hail nets. The orchard was composed of 7 year old trees, grafted on M9 rootstock, with spacings of 3.5 m × 0.45 m. The soil of the experimental field is a Latosol Bruno Aluminum - LBa, according to the Brazilian soil classification system (Santos et al., 2018). According to the Köppen-Geiger classification, the climate is 'Cfb,' constantly moist temperate with mild summer. Black anti-hail nets, with a mesh opening of 4×7 mm, 25% to 35% photosynthetic active radiation (PAR), were installed in 2010.

2.2. Treatments

Treatments evaluated were: control (plants sprayed with water); AVG (0.125 g L^{-1}, sprayed 30 d before the expected harvest date – hereafter DBEH); AVG (0.125 g L^{-1}, sprayed 30 DBEH) + ethephon (0.120 g L^{-1}, sprayed 7 DBEH); split-doses of AVG (0.0625 g L^{-1}, sprayed 30 and 20 DBEH); split-doses of AVG (0.0625 g L^{-1}, sprayed 30 and 20 DBEH) + ethephon (0.120 g L^{-1}, sprayed 7 DBEH); and half-dose of AVG (0.0625 g L^{-1}, sprayed 20 DBEH). AVG (15% active ingredient) and ethephon (24% active ingredient) were provided by ReTain® (Valent BioSciences Corporation, Libertyville, IL, USA) and Ethrel® (Lanxess Corporation, Charleston, SC, USA), respectively. The adhesive spreader Break Thru® (Evonik Corporation, Hopewell, VA, USA) at 0.01% v/v was used in the treatments. The harvests were carried out in the commercial harvest for the control treatment (February 5, 2016) and 14 d after it (February 19, 2016, late harvest).

2.3. Storage conditions and variables evaluated

Fruits were stored for 8 months under CA (1.0 kPa of O_2 and 2.0 kPa of CO_2, at a temperature of 0.5 ± 0.2°C and RH of 92 ± 5 %) in experimental mini-chambers with a capacity of 233 L. The CA conditions were established by diluting the O_2 in the storage environment with injections of N_2 and, subsequently, CO_2 until reaching the pre-established partial pressures. N_2 and CO_2 gases used came from high-pressure cylinders. The monitoring of the partial pressures of the gases, which varied due to fruit respiration, was carried out daily with the use of automatic equipment for gas control (Schelle®/Germany). When the partial pressures of CO_2 and O_2 were not adequate, the correction was carried out until they reached the partial pressures established. The excess CO_2 was corrected by injecting N_2, and O_2 consumed by breathing, with the injection of atmospheric air in the mini-chamber.

After being removed from the mini-chamber and reaching room temperature, the following attributes of the fruit were evaluated: respiratory rate (ηmol of CO_2 kg^{-1} s^{-1}); ethylene production rate (ηmol C_2H_4 kg^{-1} s^{-1}); skin color (hue angle; less red region); fruit cracking; and decay. The fruit remained for 7 days in ambient conditions (20 ± 5°C and RH of 63 ± 2%) to simulate the marketing period. After this, they were evaluated for respiratory rate (ηmol of CO_2 kg^{-1} s^{-1}); ethylene production rate (ηmol C_2H_4 kg^{-1} s^{-1}); skin color (hue angle; less red region); flesh firmness (N); texture [force to break the skin (N) and to penetrate the flesh (N)]; soluble solids (SS; %); titratable acidity (TA; % malic acid); mealiness; fruit cracking; decay; total antioxidant activity (TAA; DPPH and ABTS methods, mol TEAC kg^{-1}); total phenolic compounds (TPC; g EAG kg^{-1}) of the skin and flesh; and content of the phenolic compounds of the skin (chlorogenic acid, floridizine, epicatechin, and procyanidin B1, in mg kg^{-1}).

2.3.1. Physicochemical attributes of the fruit

The assessment of the respiratory rate (ηmol CO_2 kg^{-1} s^{-1}); ethylene production rate (ηmol C_2H_4 kg^{-1} s^{-1}); skin color (hue angle; less red region); flesh firmness (N); texture attributes (N; force required to break the skin and to penetrate the flesh); TA (% malic acid); and SS (%) were performed according to the methodology described in Soethe et al. (2019).

Respiratory and ethylene production rates were quantified by gas chromatography. Fruits of each repetition were placed in 4.1 L containers, with hermetic closure. Respiratory and ethylene production rates were obtained by the concentration of CO_2 and C_2H_4, respectively, inside the container, after 30 min of closing the containers containing the fruits. After this period, using a 1.0 mL plastic syringe, three samples of the atmosphere were collected from the free space in these containers, which were injected into a gas chromatograph, Varian®, model CP-3800 (Palo Alto, USA), equipped with a 3 m long Porapak N® column (80-100 mesh), methanator, and flame ionization detector. Column, detector, methanator, and inlet temperatures were 70; 250; 380; and 130°C, respectively. The nitrogen, hydrogen, and synthetic air fluxes used were 70; 30; and 300 mL min^{-1}, respectively.

The skin color (less red region) was evaluated in terms of hue angle values (h°) with the aid of a Minolta® colorimeter model CR 400 (Konica, Tokyo, Japan). The values of h° present the following correspondences regarding the surface colors of the plant tissue: 0°/red,

90º/yellow, 180º/green, and 270º/blue. The readings were carried out in the equatorial region of the fruits.

Flesh firmness (N) was determined in the equatorial region of the fruits, on two opposite surfaces, after removing a small portion of the epidermis, with the aid of an electronic penetrometer (GÜSS Manufacturing Ltd, Cape Town 48, South Africa) equipped with 11 mm diameter tip. Texture attributes were analyzed with a TAXT plus® electronic texturometer (Stable Micro Systems Ltd, Surrey, UK), in terms of forces required for skin breakage (N) and flesh penetration (N), using a tip with 2 mm in diameter, which was introduced into the flesh at a depth of 10 mm, with pre-test, test, and post-test speeds of 30, 3, and 40 mm s^{-1}, respectively.

The values of TA (% malic acid) were obtained through a 10 mL sample of juice, obtained by processing the fruits in a centrifuge. This sample was diluted in 90 mL of distilled water and titrated with 0.1N NaOH solution to pH 8.1. For sample titration, an automatic TitroLine® easy titrator from SCHOTT Instruments (Mainz, Germany) was used.

The SS contents (%) were determined in a digital refractometer model PR201α (Atago®, Tokyo, Japan), using an aliquot of the juice obtained by processing the fruits.

Mealiness (%) was determined by subjective visual assessment and quantification of fruit that showed symptoms such as dry flesh and little juiciness (Stanger et al., 2018). Fruit cracking (%) was established by counting the fruit with cracked skin or flesh; and decay, by counting the fruit with internal or external lesions caused by pathogens (Steffens et al., 2005).

2.3.2. Total antioxidant activity, total phenolic compounds, and content of chlorogenic acid, floridizine, epicatechin, and procyanidin B1

The analyses of TAA, TPC, and the contents of chlorogenic acid, floridizine, epicatechin, and procyanidin B1 were carried out adopting the methodology found in Stanger et al. (2018), with adaptations. The reagents 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchromium -2-carboxylic acid (Trolox), Folin-Ciocalteau, sodium acetate, and potassium persulfate were purchased from Sigma Chemical Co. (St. Louis, MO, USA), and were of analytical grade (PA). Gallic acid, sodium carbonate, acetone, and ethyl alcohol were obtained from Vetec® (Rio de Janeiro, Brazil), and were of analytical grade (PA). The chlorogenic acid, phloridizin, epicatechin, and procyanidin B1 standards, as well as the solvents acetonitrile, acetic acid, and methanol were purchased from Sigma Chemical Co. (St. Louis, MO, USA), all with HPLC grade purity.

TAA and TPC were analyzed in two parts of the fruit (skin and flesh). The skin of the entire surface of the fruit was removed with a cutting blade (1 mm thick). The flesh sample was removed by means of a longitudinal slice, about one centimeter from the middle portion of the fruit, discarding the endocarp region and conserving each side of the slice. The flesh samples were processed with a vertical crusher, Philips Walita, model RI1364 (Varginha, Brazil), and the skin samples were macerated in a mortar with liquid nitrogen.

To obtain the extracts used in the quantification of TPC and TAA, 5 and 2.5 g of samples were used for the flesh and skin, respectively, which were homogenized in 10 mL of methanol/distilled water (50:50, v /v), with subsequent homogenization in an Ultra-Turrax, Heidolph brand, model D-91126 (Schwabach, Germany) and placed to rest for 60 min at room temperature (20°C). Then, the samples were centrifuged in an Eppendorf centrifuge, model 5810R (Hamburg, Germany) at 4°C for 20 min at 10000 rpm. The supernatant was filtered through a 25 ml volumetric flask. From the residue of the first extraction, 10 mL of acetone/distilled water (70:30, v/v) was added, homogenized, and left to rest for 60 min at room temperature. After this period, it was centrifuged again (4°C) for 20 min at 10000 rpm. The supernatant was transferred to the volumetric flask (containing the first supernatant) and made up to 25 ml with distilled water. Extracts were reserved for TPC and TAA analysis.

The determination of TPC was performed using the Folin-Ciocalteau reagent. The standard curve was obtained with gallic acid at concentrations of 0, 10, 30, 50, 70, 90, and 100 ppm. For analysis, 2.5 ml of Folin-Ciocalteau/distilled water (1:3, v/v), 0.5 ml of sample, and 2.0 ml of 10% sodium carbonate solution were added. The tubes were shaken, and incubated for one hour in the dark. The reading was performed in a microplate reader, model EnSpire (PerkinElmer, USA) at a wavelength (λ) of 765 nm. The results were expressed in mg of gallic acid equivalents per 100 g of fresh mass of the sample (g EAG kg^{-1} FM).

The determination of TAA was based on extinction and the absorption of DPPH (2,2-diphenyl-1-picryl hydrazyl) and ABTS (2,2-azinobis-3-ethylbenzothiazolin-6-sulfonic acid) radicals. In a dark environment, 100 μL of sample were pipetted and mixed with 3.900 μL of DPPH radical. The tubes were shaken and left to react for 30 min. The reading was performed at λ=515 nm, and the results expressed in μmol of Trolox equivalent100 g^{-1} of fresh mass of the sample. In a dark environment, 30 μL of sample were pipetted and mixed with 3.000 μL of ABTS radical. The reading was performed after a 6-minute reaction at λ=734 nm, and the results were expressed in μmol of Trolox equivalent g^{-1} of fresh mass of the sample.

For the quantification of chlorogenic acid, floridizine, epicatechin, and procyanidin B1, the sample preparation was the same used for TPC and TAA. Each sample was transferred to a beaker with methanol/ultrapure water (70:30, v/v), in the proportion of 1:1 (w/v), where they were homogenized using a Heidolph Ultra-Turrax, model D-91126 (Schwabach, Germany). Filtration was performed using a quantitative filter under vacuum and then through a 0.45 μm syringe filter, Kasvi brand (Curitiba, Brazil). The final sample remained stored at -20°C until analysis.

Quantification was performed using a high performance liquid chromatograph (HPLC), with a Shimadzu chromatograph (Tokyo, Japan), equipped with an SCL-10Avp controller, FCV-10ALvp quaternary mixer, LC-10ADvp pump, SIL 10-ADvp, SPD-10AVp ultraviolet detector, and CLASS VP 6.14 software. C18 analytical column (250×4.6 mm; particle size, 5 μm), Restek brand (Bellefonte, USA) was used. The mobile phase was acetic acid/ultrapure water (6:94, v/v) in 2 mM sodium acetate buffer (solvent A, pH 2.55, v/v) and acetonitrile (solvent B). The gradient program was as follows: 0% to 15% B in 45 min, 15% to 30% B in 15 min, 30% to 50% B in 5 min, and 50% to 100% B in 5 min. The return time to the initial condition was 10 min. The flow rate was 1.0 mL min^{-1} for a total run time of 80 min. The detector was set at λ=280 nm and the injection volume at 20 μL for all samples. All standards were dissolved in methanol.

The identification of phenolic compounds was based on retention times of the standards: procyanidin B1 = 10.9 min; chlorogenic acid = 22.7 min; epicatechin = 35.2 min; and phloridizin = 67.0 min. Confirmation of identity was obtained by adding the internal standard to the samples and comparing them to the same sample without the addition of the internal standard. The analyte concentration was calculated according to calibration curves derived from the corresponding pure standard phenolic compound, at concentrations between 0-100 mg g^{-1}. All samples were prepared and analyzed in duplicate.

Analyses of chlorogenic acid, floridizine, epicatechin, and procyanidin B1 were performed on the fruit skin of those treated with the control (plants sprayed with water), single-dose of AVG (0.125 g L^{-1}, sprayed 30 DBEH), split-doses of AVG (0.0625 mg L^{-1} + 0.0625 mg L^{-1}, sprayed 30 and 20 DBEH), and half-dose of AVG (0.0625 mg L^{-1}, sprayed 20 DBEH) treatments on two harvest dates [commercial harvest of the control treatment (harvest 1) and 14 d after (harvest 2)].

2.4. Physicochemical attributes at harvest

At harvest, in order to indicate the fruit maturation stage, the following attributes were evaluated: iodine starch index; SS; TA content; flesh firmness; and red color index (RCI). SS, TA content, and flesh firmness were analyzed using the methodology described by

Table 1
Physicochemical attributes of 'Baigent' apples postharvest (initial analysis).

Treatments	Commercial harvest Feb 5	Late harvest Feb 19
	Iodine starch index (1-5)	
Control	3.3	4.7
AVG 0.125 g L^{-1} (30 DBEH*)	2.9	3.6
AVG 0.125 g L^{-1} (30 DBEH) + ethephon 0.120 g L^{-1} (7 DBEH)	3.8	4.5
AVG 0.0625 g L^{-1} (30 DBEH) + AVG 0.0625 g L^{-1} (20 DBEH)	2.7	3.3
AVG 0.0625 g L^{-1} (30 DBEH) + AVG 0.0625 g L^{-1} (20 DBEH) + ethephon 0.120 g L^{-1} (7 DBEH)	3.5	3.6
AVG 0.0625 g L^{-1} (20 DBEH)	3.0	3.5
	Soluble solids (%)	
Control	11.8	12.2
AVG 0.125 g L^{-1} (30 DBEH)	10.2	10.6
AVG 0.125 g L^{-1} (30 DBEH) + ethephon 0.120 g L^{-1} (7 DBEH)	11.3	12.5
AVG 0.0625 g L^{-1} (30 DBEH) + AVG 0.0625 g L^{-1} (20 DBEH)	10.0	11.0
AVG 0.0625 g L^{-1} (30 DBEH) + AVG 0.0625 g L^{-1} (20 DBEH) + ethephon 120 g L^{-1} (7 DBEH)	10.6	11,9
AVG 0.0625 g L^{-1} (20 DBEH)	9.8	11.0
	Titratable acidity (% malic acid)	
Control	0.189	0.165
AVG 0.125 g L^{-1} (30 DBEH)	0.161	0.144
AVG 0.125 g L^{-1} (30 DBEH) + ethephon 0.120 g L^{-1} (7 DBEH)	0.176	0.175
AVG 0.0625 g L^{-1} (30 DBEH) + AVG 0.0625 g L^{-1} (20 DBEH)	0.176	0.159
AVG 0.0625 g L^{-1} (30 DBEH) + AVG 0.0625 g L^{-1} (20 DBEH) + ethephon 0.120 g L^{-1} (7 DBEH)	0.181	0.172
AVG 0.0625 g L^{-1} (20 DBEH)	0.239	0.162
	Flesh firmness (N)	
Control	81.2	69.8
AVG 0.125 g L^{-1} (30 DBEH)	79.1	73.7
AVG 0.125 g L^{-1} (30 DBEH) + ethephon 0.120 g L^{-1} (7 DBEH)	78.5	75.6
AVG 0.0625 g L^{-1} (30 DBEH) + AVG 0.0625 g L^{-1} (20 DBEH)	76.1	74.2
AVG 0.0625 g L^{-1} (30 DBEH) + AVG 0.0625 g L^{-1} (20 DBEH) + ethephon 0.120 g L^{-1} (7 DBEH)	76.7	76.3
AVG 0.0625 g L^{-1} (20 DBEH)	80.4	71.8
	Red color index of the skin (1-4)	
Control	3.9	3.5
AVG 0.125 g L^{-1} (30 DBEH)	2.4	2.6
AVG 0.125 g L^{-1} (30 DBEH) + ethephon 0.120 g L^{-1} (7 DBEH)	3.7	3.5
AVG 0.0625 g L^{-1} (30 DBEH) + AVG 0.0625 g L^{-1} (20 DBEH)	2.6	3.0
AVG 0.0625 g L^{-1} (30 DBEH) + AVG 0.0625 g L^{-1} (20 DBEH) + ethephon 0.120 g L^{-1} (7 DBEH)	3.0	3.5
AVG 0.0625 g L^{-1} (20 DBEH)	2.5	3.2

* DBEH: days before the expected harvest date of control treatment.

Steffens et al. (2006) and Soethe et al. (2021). The iodine starch index was evaluated based on a scale from 1 to 5 in which 1 indicates the maximum starch content and 5, the fully hydrolyzed starch (Stanger et al., 2017). The RCI was determined using the methodology described in Soethe et al. (2021), with adaptations. Each fruit was individually evaluated in relation to the red color of its skin and classified using a scale from 1 to 4, in which 1 represented a red-pigmented surface of 0% to 25% of the total fruit surface; 2, from 26% to 50%; 3, from 51% to 75%; and 4, from 76% to 100%. The index was obtained by multiplying the number of fruit classified in each index by the index number; then, the result of the sum of these multiplications was divided by the number of fruits assessed in each sample.

2.5. Experimental design and statistical analysis

The experiment was carried out with randomized blocks under a factorial design (6×2 – six treatments and two harvest dates, except for the analyses of TAA, TPC, and phenolic compounds content, which considered four treatments and two harvest dates) with four repetitions of 20 fruit each. Before the statistical analysis was carried out, the incidence data (percentage of mealiness, cracks, and rotten fruit) were transformed into arc sin $(x + 1/100)^{0.5}$ percentage values. Data were submitted to analysis of variance and the treatment means compared by the Tukey's test ($p < 0.05$). On the fruit skin, the variable TAA was submitted to Pearson's correlation analysis ($p < 0.01$) with the levels of TPC. All data were analyzed using the SAS software (SAS Institute, 2002).

3. Results and Discussion

3.1. Fruit quality

The physicochemical attributes at harvest of 'Baigent' apples are shown in Table 1.

The apples did not show significant differences ($p<0.05$) among treatments for respiratory rate (Table 2) after 8 months of storage in CA, either at the time when they were removed from CA storage or after 7 days in ambient conditions. The absence of difference between treatments for respiratory rate may be related to the effect of the CA condition on fruit respiration, as, due to the conditions of low partial pressures of O_2 (1.0 kPa) and high partial pressures of CO_2 (2.0 kPa), there is a reduction in cellular respiration (Steffens et al., 2007; Weber et al., 2013).

There was an interaction between the pre-harvest treatments and harvest dates for the ethylene production rate, both at removal from storage and after 7 days in ambient conditions (Table 2). At removal from CA storage, on the day of commercial harvest, apples showed no difference between treatments; however, when harvested 14 d later, apples with the application of AVG at the dose of 0.125 g L^{-1} showed a lower rate of ethylene production when compared to the control treatment. After 7 days in ambient condition, apples harvested on the commercial harvest date also showed no difference among treatments for the ethylene production rate. Nevertheless, apples harvested late, when submitted to AVG application, regardless of the dose, forms of application, and whether or not it was combined with ethephon, pre-

Table 2
Respiratory rate (*n*mol of CO_2 kg^{-1} s^{-1}) and ethylene production rate (*n*mol C_2H_4 kg^{-1} s^{-1}) of 'Baigent' apples after storage for 8 months in controlled atmosphere (CA; 1.0 kPa O_2 + 2.0 kPa CO_2/0.5°C/92 % RH), at removal from CA storage, and after 7 days in ambient conditions (20±1°C and 65±5 % RH).

Treatments	Commercial harvest Feb 5	Late harvest Feb 19	Mean	Commercial harvest Feb 5	Late harvest Feb 19	Mean
	Respiratory rate			Ethylene production rate		
			At removal from storage			
Control	72.1	178.5	0.125.3^{ns}	0.001 Ab	0.087 Aa**	.
AVG 0.125 g L^{-1} (30 DBEH*)	74.6	153.7	114.2	0.000 Ab	0.035 Ba	.
AVG 0.125 g L^{-1} (30 DBEH) + ethephon 0.120 g L^{-1} (7 DBEH)	76.9	175.1	126.0	0.000 Ab	0.103 Aa	.
AVG 0.0625 g L^{-1} (30 DBEH) + AVG 0.0625 g L^{-1} (20 DBEH)	72.9	175.8	124.4	0.000 Ab	0.096 Aa	.
AVG 0.0625 g L^{-1} (30 DBEH) + AVG 0.0625 g L^{-1} (20 DBEH) + ethephon 0.120 g L^{-1} (7 DBEH)	64.5	170.7	117.6	0.000 Ab	0.086 Aa	.
AVG 0.0625 g L^{-1} (20 DBEH)	72.9	171.9	122.4	0.000 Ab	0.086 Aa	.
Mean	72.3b	171.0a		.	.	.
CV (%)	3,7			21.0		
	7 days after being in ambient conditions					
Control	199.9	88.8	144.4^{ns}	0.032 Ab	0.101 Aa	.
AVG 0.125 g L^{-1} (30 DBEH)	234.9	85.6	160.3	0.033 Aa	0.003 Cb	.
AVG 0.125 g L^{-1} (30 DBEH) + ethephon 0.120 g L^{-1} (7 DBEH)	215.0	124.9	169.9	0.038 Aa	0.044 Ba	.
AVG 0.0625 g L^{-1} (30 DBEH) + AVG 0.0625 g L^{-1} (20 DBEH)	257.1	105.7	181.4	0.050 Aa	0.017 BCb	.
AVG 0.0625 g L^{-1} (30 DBEH) + AVG 0.0625 g L^{-1} (20 DBEH) + ethephon 0.120 g L^{-1} (7 DBEH)	244.9	100.0	172.5	0.047 Aa	0.041 Ba	.
AVG 0.0625 g L^{-1} (20 DBEH)	190.3	83.6	136.9	0.031 Aa	0.021 BCb	.
Mean	223.7 a			.98.1 b	.	
CV (%)	27.5			33.5		

* DBEH: days before the expected harvest date of control treatment. **Means not followed by the same letter, uppercase in vertical and lowercase in horizontal, differ from each other by the Tukey test ($p < 0.05$). ^{ns}: non-significant difference.

sented lower ethylene production rates than the control. Among the treatments, the lowest rate of ethylene production was observed in the fruit that received pre-harvest application of AVG in a single-dose of 0.125 g L^{-1}, despite not differing from the other forms of application of AVG, split-doses (0.0625 g L^{-1} + 0.0625 g L^{-1}) or half-dose (0.0625 g L^{-1}). Brackmann et al. (2015a) also observed a lower rate of ethylene production, after being stored in CA, in apples that received pre-harvest application of AVG compared to the control. In accordance with Wendt et al. (2020), the AVG blocks the production of ACC, by inhibiting its synthase, reducing the production of ethylene in the fruit. Steffens et al. (2006), in a work carried out with Gala apples, observed that the application of AVG followed by ethephon reduces the ethylene production rate as well. These results may be related to the fact that AVG prevents the autocatalytic production of ethylene which would be triggered by the ethylene released by ethephon (Iqbal et al., 2017).

There was no interaction between the pre-harvest treatments and harvest dates for the fruit skin color. At removal from storage, fruit that had received only pre-harvest applications of AVG, single-dose (0.125 g L^{-1}), split-dose (0.0625 g L^{-1} + 0.0625 g L^{-1}), or half-dose (0.0625 g L^{-1}), presented, in comparison to the control, a greener skin background color (higher value of h°). Apples treated with ethephon after the AVG application showed intermediate values, which did not differ from the AVG and control treatments. After 7 days in ambient conditions, all pre-harvest applications of AVG, regardless of the application or not of ethephon, showed a greener skin background color when compared to the control (Table 3). Steffens et al. (2005) observed, when studying Gala apples, fruit with greener background color at the end of the storage period in CA, considering those which had received pre-harvest application of AVG combined or not with ethephon. According to Soethe et al. (2021), the effect of AVG on greener background color is related to the lower ethylene production rate of these fruit and, consequently, lower activity of chlorophyllase enzymes, which act in the degradation of chlorophylls (Brackmann et al., 2009).

There was no interaction between the pre-harvest treatment and harvest dates for the SS and TA values (Table 4). Apples with pre-harvest applications of AVG at 0.125 g L^{-1} and split-doses (0.0625 g L^{-1} + 0.0625 g L^{-1}) showed lower SS values compared to the control, even though these values did not differ from those found for the treatment using half the recommended dose of AVG (0.0625 g L^{-1}) and AVG combined with ethephon. Pre-harvest applications of AVG only (0.125 g L^{-1}; 0.0625 g L^{-1} + 0.0625 g L^{-1}; and 0.0625 g L^{-1}) provided the fruit with higher TA when compared to the control treatment, while the ones treated with AVG combined with ethephon presented intermediate results, not differing from the other treatments. According to Brackmann et al. (2015a), higher SS content is related to the reduction in flesh firmness caused by the hydrolysis of the cell wall. However, lower levels of SS in apples treated with AVG may be associated with a reduction in the rate of ethylene production, resulting in less starch hydrolysis (Brackmann et al., 2015a). Higher TA in apples treated with AVG was also observed by Aglar et al. (2016). According to Brackmann et al. (2009), AVG delays fruit ripening and, thereby, inhibits the degradation of organic acids, maintaining higher TA values.

After 8 months of storage in CA, there was no interaction between the pre-harvest treatments and harvest dates for flesh firmness and texture attributes (force to break the skin and penetrate the flesh) (Table 5). Flesh firmness and texture attributes were higher in apples that received AVG pre-harvest application, regardless of dose, application form, and combination with ethephon, compared to the control treatment. Brackmann et al. (2015b) also observed greater flesh firmness in 'Baigent' apples treated with AVG after storage in CA, regardless of the combination with ethephon. Greater firmness of the flesh may be related to the lower activity of ACC oxidase and ethylene production rate in fruit treated with AVG (Table 2), since, according to Brackmann et al. (2015a), ethylene acts as an activator of enzymes that degrade cell wall components, which results in lower firmness of the flesh. With the application of AVG, there is a delay in ripening due to the

Table 3
Hue angle (*h*°; background color) of 'Baigent' apples after 8 months of storage in controlled atmosphere (CA;1.0 kPa O_2 + 2.0 kPa CO_2/0.5°C/92 % RH), at the time of removal from CA storage, and after 7 days in ambient conditions (20±1°C and 65±5 % RH).

Treatments	Commercial harvest Feb 5	Late harvest Feb 19	Mean	Commercial harvest Feb 5	Late harvest Feb 19	Mean
	At removal from storage			After 7 days in ambient conditions		
Control	91.8	74.9	83.3 B**	76.8	83.1	79.9 B
AVG 0.125 g L^{-1} (30 DBEH*)	106.2	92.1	99.2 A	95.9	99.7	97.8 A
AVG 0.125 g L^{-1} (30 DBEH) + ethephon 0.120 g L^{-1} (7 DBEH)	104.6	75.7	90.2 AB	91.6	87.5	89.6 A
AVG 0.0625 g L^{-1} (30 DBEH) + AVG 0.0625 g L^{-1} (20 DBEH)	106.2	88.5	97.4 A	91.2	96.0	93.6 A
AVG 0.0625 g L^{-1} (30 DBEH) + AVG 0.0625 g L^{-1} (20 DBEH) + ethephon 0.120 g L^{-1} (7 DBEH)	105.0	75.4	90.2 AB	89.0	87.7	89.2 AB
AVG 0.0625 g L^{-1} (20 DBEH)	106.8	87.3	97.0 A	93.5	93.1	93.3 A
Mean	103.4 a	82.3 b		89.7 a	91.6 a	
CV (%)	6.6			5.7		

* DBEH: days before the expected harvest date of control treatment. **Means not followed by the same letter, uppercase in vertical and lowercase in horizontal, differ from each other by the Tukey test ($p < 0.05$).

Table 4
TA and SS of 'Baigent' apples after 8 months of storage in controlled atmosphere (CA; 1.0 kPa O_2 + 2.0 kPa CO_2/0.5°C/92 % RH) plus 7 days in ambient conditions (20±1°C and 65±5 % RH).

Treatments	Commercial harvest Feb 5	Late harvest Feb 19	Mean
	Soluble solids (SS; %)		
Control	12.8	12.7	12.8 A**
AVG 0.125 g L^{-1} (30 DBEH*)	10.8	11.6	11.2 B
AVG 0.125 g L^{-1} (30 DBEH) + ethephon 0.120 g L^{-1} (7 DBEH)	11.3	12.2	11.7 AB
AVG 0.0625 g L^{-1} (30 DBEH) + AVG 0.0625 g L^{-1} (20 DBEH)	11.5	11.5	11.5 B
AVG 0.0625 g L^{-1} (30 DBEH) + AVG 0.0625 g L^{-1} (20 DBEH) + ethephon 0.120 g L^{-1} (7 DBEH)	11.7	12.3	12.0 AB
AVG 0.0625 g L^{-1} (20 DBEH)	11.7	12.0	11.9 AB
Mean	11.6 a	12.1 a	
CV (%)	5.5		
	Titratable acidity (TA; % malic acid)		
Control	0.223	0.199	0.211 B
AVG 0.125 g L^{-1} (30 DBEH)	0.270	0.214	0.236 A
AVG 0.125 g L^{-1} (30 DBEH) + ethephon 0.120 g L^{-1} (7 DBEH)	0.232	0.208	0.220 AB
AVG 0.0625 g L^{-1} (30 DBEH) + AVG 0.0625 g L^{-1} (20 DBEH)	0.257	0.213	0.235 A
AVG 0.0625 g L^{-1} (30 DBEH) + AVG 0.0625 g L^{-1} (20 DBEH) + ethephon 0.120 g L^{-1} (7 DBEH)	0.239	0.219	0.227 AB
AVG 0.0625 g L^{-1} (20 DBEH)	0.253	0.222	0.237 A
Mean	0.244 a	0.213 b	
CV (%)	5.4		

* DBEH: days before the expected harvest date of control treatment. **Means not followed by the same letter, uppercase in vertical and lowercase in horizontal, differ from each other by the Tukey test ($p < 0.05$).

inhibition of ethylene synthesis and lower activity of the pectin methyl esterase and polygalacturonase enzymes, which are responsible for flesh softening (Wendt et al., 2020).

There was an interaction between the pre-harvest treatments and harvest dates for the incidence of cracks. In the first harvest, no cracks were observed in any of the treatments. In the second harvest, apples that were treated only with AVG did not present cracked fruit (Table 6). According to Steffens et al. (2005), the effect of AVG on controlling the occurrence of cracks is associated with its action in reducing the synthesis of ethylene, as it can increase cracking incidence by accelerating fruit ripening. The authors also attribute the lower occurrence of cracked fruit to the lower degradation of pectin of the flesh cell wall.

Decay was lower in the fruit that received application of AVG in a single-dose of 0.125 g L^{-1}, both at removal from CA storage and after 7 days in ambient condition, being higher in the apples that were treated with ethephon after AVG, regardless of the form (Table 6). The other pre-harvest applications, split-doses (0.0625 g L^{-1} + 0.0625 g L^{-1}) and half-dose (0.0625 g L^{-1}), did not differ between the best and the worst treatments. Possibly, the fruit treated with AVG in a single-dose of 0.125 g L^{-1} had lower decay due to the less advanced stage of ripeness at the time of harvest, as less ripe fruit are less susceptible to rot than ripe fruit because the latter are less resistant to flesh penetration and development of pathogens. According to Brackmann et al. (2015a), the decay rate of fruit that were treated with ethephon after the application of AVG is related to the transformation of ethephon into ethylene, which promotes fruit ripening and increases susceptibility to pathogens.

Pre-harvest applications of AVG, either a single-dose of 0.125 g L^{-1} or split-doses (0.0625 g L^{-1} + 0.0625 g L^{-1}), caused a lower incidence of mealiness when compared to the control, however, those did not differ from the application of half-dose (0.0625 g L^{-1}) and AVG combined with ethephon (Table 6). Soethe et al. (2019) demonstrated that pre-harvest application of AVG can reduce the incidence of mealiness in apples. The lower occurrence of this disturbance can be attributed to the lower pectin degradation in the wall of flesh cells (Soethe et al., 2019), thus, reducing the cohesive force between cells, which causes them to detach from each other and not break during chewing.

Table 5
Flesh firmness and texture attributes of 'Baigent' apples after 8 months of storage in controlled atmosphere (CA; 1.0 kPa O_2 + 2.0 kPa CO_2/0.5°C/92 % RH) plus 7 days in ambient conditions (20±1°C and 65±5 % RH).

Treatments	Commercial harvest Feb 5	Late harvest Feb 19	Mean
	Flesh firmness (N)		
Control	71.9	61.8	66.8 B**
AVG 0.125 g L^{-1} (30 DBEH*)	81.9	69.8	75.8 A
AVG 0.125 g L^{-1} (30 DBEH) + ethephon 0.120 g L^{-1} (7 DBEH)	80.3	64.9	72.6 A
AVG 0.0625 g L^{-1} (30 DBEH) + AVG 0.0625 g L^{-1} (20 DBEH)	79.1	69.3	74.2 A
AVG 0.0625 g L^{-1} (30 DBEH) + AVG 0.0625 g L^{-1} (20 DBEH) + ethephon 0.120 g L^{-1} (7 DBEH)	79.8	68.9	74.3 A
AVG 0.0625 g L^{-1} (20 DBEH)	81.0	68.8	74.9 A
Mean	79.0 a	67.2 b	
CV (%)	3.6		
	Force to break the skin (N)		
Control	13.1	11.8	12.5 B
AVG 0.125 g L^{-1} (30 DBEH)	14.9	13.6	14.3 A
AVG 0.125 g L^{-1} (30 DBEH) + ethephon 0.120 g L^{-1} (7 DBEH)	14.9	13.2	14.1 A
AVG 0.0625 g L^{-1} (30 DBEH) + AVG 0.0625 g L^{-1} (20 DBEH)	14.7	13.2	13.9 A
AVG 0.0625 g L^{-1} (30 DBEH) + AVG 0.0625 g L^{-1} (20 DBEH) + ethephon 0.120 g L^{-1} (7 DBEH)	14.1	13.0	13.5 A
AVG 0.0625 g L^{-1} (20 DBEH)	14.3	13.2	13.8 A
Mean	14.3 a	13. b	
CV (%)	4.2		
	Force to penetrate the flesh (N)		
Control	2.9	2.5	2.7 B
AVG 0.125 g L^{-1} (30 DBEH)	3.5	3.0	3.2 A
AVG 0.125 g L^{-1} (30 DBEH) + ethephon 0.120 g L^{-1} (7 DBEH)	3.3	2.8	3.1 A
AVG 0.0625 g L^{-1} (30 DBEH) + AVG 0.0625 g L^{-1} (20 DBEH)	3.2	2.8	3.0 A
AVG 0.0625 g L^{-1} (30 DBEH) + AVG 0.0625 g L^{-1} (20 DBEH) + ethephon 0.120 g L^{-1} (7 DBEH)	3.3	2.8	3.1 A
AVG 0.0625 g L^{-1} (20 DBEH)	3.3	2.9	3.1 A
Mean	3.3 a	2.8 b	
CV (%)	4.7		

* DBEH: days before the expected harvest date of control treatment. **Means not followed by the same letter, uppercase in vertical and lowercase in horizontal, differ from each other by the Tukey test ($p < 0.05$).

Table 6
Incidence (%) of fruit cracking, decay, and mealiness of 'Baigent' apples after 8 months of storage in controlled atmosphere (CA; 1.0 kPa O_2 + 2.0 kPa CO_2/0.5°C and 92 % RH). Cracks were assessed at removal from CA storage; decay, at removal from CA storage and after 7 days in ambient condition (20±1°C and 65±5 % RH); and mealiness, after 7 days in ambient condition.

Treatments	Commercial harvest Feb 5 At removal from storage	Late harvest Feb 19	Mean	Commercial harvest Feb 5	Late harvest Feb 19	Mean
		Fruit cracking			Decay	
Control	0.0 Aa**	8.7 ABa	.	3.3	19.2	11.3 AB
AVG 0.125 g L^{-1} (30 DBEH*)	0.0 Aa	0.0 Ba	.	0.0	8.4	4.2 B
AVG 0.125 g L^{-1} (30 DBEH) + ethephon 0.120 g L^{-1} (7 DBEH)	0.0 Ab	12.9 Aa	.	8.7	12.5	10.6 AB
AVG 0.0625 g L^{-1} (30 DBEH) + AVG 0.0625 g L^{-1} (20 DBEH)	0.0 Aa	0.0 Ba	.	7.0	18.3	12.7 AB
AVG 0.0625 g L^{-1} (30 DBEH) + AVG 0.0625 g L^{-1} (20 DBEH) + ethephon 0.120 g L^{-1} (7 DBEH)	0.0 Aa	6.7 ABa	.	5.1	32.3	18.7 A
AVG 0.0625 g L^{-1} (20 DBEH)	0.0 Aa	0.0 Ba	.	7.2	9.9	8.6 AB
Mean	.	.		5.2 b	16.9 a	
CV (%)	39.4			21.0		
	After 7 days in ambient conditions					
	Mealiness		Decay			
Control	21.4	43.1	32.2 A	3.3	42.3	22.8 A
AVG 0.125 g L^{-1} (30 DBEH)	0.0	0.0	0.0 B	0.0	13.0	6.5 B
AVG 0.125 g L^{-1} (30 DBEH) + ethephon 0.120 g L^{-1} (7 DBEH)	0.0	28.7	14.4 AB	8.7	41.4	25.1 A
AVG 0.0625 g L^{-1} (30 DBEH) + AVG 0.0625 g L^{-1} (20 DBEH)	0.0	12.2	6.1 B	7.0	30.5	18.8 AB
AVG 0.0625 g L^{-1} (30 DBEH) + AVG 0.0625 g L^{-1} (20 DBEH) + ethephon 0.120 g L^{-1} (7 DBEH)	4.4	27.8	16.1 AB	5.1	59.3	32.2 A
AVG 0.0625 g L^{-1} (20 DBEH)	7.4	12.6	10.0 AB	7.2	22.7	15.0 AB
Mean	5.5 b	20.7 a		5.2 b	34.9 a	
CV (%)	5.6			17.8		

* DBEH: days before the expected harvest date of control treatment. **Means not followed by the same letter, uppercase in vertical and lowercase in horizontal, differ from each other by the Tukey test ($p < 0.05$).

As for the harvest date, at removal from storage, those fruit harvested late, when compared to the ones harvested commercially, showed an increase in the ethylene production rates for all treatments (Table 2). After 7 days in ambient conditions, late-harvested fruit which received only pre-harvest application of AVG [single-dose (0.125 g L^{-1}), split-doses (0.0625 g L^{-1} + 0.0625 g L^{-1}), or half-dose (0.0625 g L^{-1})] showed a reduction in the ethylene production rate. Differently, late-harvested fruit from the control treatment showed an increase in the ethylene production rate, in relation to those from the commercial harvest. Late harvest fruit showed a higher degree of yellowing of the skin, and lower TA, flesh firmness, force to penetrate the flesh, and force to break the skin values (Tables 3, 4, and 5). Furthermore, there was an increase in the incidence of rot and mealiness, which indicates an advanced stage of fruit ripening (Table 6), and the time of harvest was found to not influence the SS content after storage (Table 4).

The effect of AVG on delay the loss of fruit quality during storage in CA of 'Baigent' apples produced under anti-hail nets is very similar to that obtained in studies carried out with apples produced in full sunlight (Steffens et al., 2005; Brackmann et al., 2015a, b; Aglar et al., 2016). Furthermore, it was evident in this study that the split-dose AVG application does not interfere negatively in the delay in fruit ripening. This result is very important for the production of 'Baigent' apples, since, for apples of this cultivar, the split application of AVG does not compromise the development of the red color of the epidermis (Soethe et al., 2021), an important quality parameter of the fruit.

3.2. Total antioxidant activity, total phenolic compounds, and content of chlorogenic acid, floridizine, epicatechin, and procyanidin B1

A positive and significant correlation ($p < 0.01$) was observed between TPC content and TAA (DPPH and ABTS methods), both in the skin and flesh of the fruit. In the flesh, a correlation coefficient of 0.70 was obtained for the DPPH method and 0.92 for the ABTS. In the skin, a correlation coefficient of 0.94 was obtained for the two methods. Positive linear correlation between TPC and TAA in apples was also reported by Stanger et al. (2017; 2018), who demonstrated that phenolic compounds are important contributors to TAA.

In the flesh, there was no difference between the pre-harvest treatments for the content of TPC and TAA, by both the DPPH and the ABTS methods after 8 months of storage in CA and after 7 days in ambient conditions (Table 7). The lack of differences between treatments for TPC and TAA can be attributed to the synergistic effects that different phenolic compounds have on TAA (Bolling, Chen, & Chen, 2013). According to Nimbolkar et al. (2016), fruit antioxidant activity is due to the action of a variety of compounds which are degraded or synthesized during storage in response to biotic and abiotic stresses.

There was no interaction between pre-harvest treatments and harvest dates for TPC and TAA values quantified in the skin. The pre-harvest application of AVG in a single-dose of 0.125 g L^{-1}, when compared to the control, allowed for lower content of TPC in the skin. The other forms of pre-harvest applications of AVG presented intermediate values and did not differ from AVG treatments with a single-dose of 0.125 g L^{-1} and control (Table 8). These results show that pre-harvest applications of AVG in split-doses and half-dose of 0.0625 g L^{-1} do not negatively affect the content of TPC in the fruit skin. According to Ozturk et al. (2015), the decrease in the content of bioactive compounds by AVG may be because it inhibits ethylene synthesis, and ethylene positively regulates the activity of phenylalanine ammonia-lyase (PAL) (Xi et al., 2013), which is the primary enzyme in the biosynthetic route to phenolic compounds.

The TAA, quantified using the DPPH and ABTS methods, was lower in the fruit treated with AVG, regardless of the form of application and combination or not with ethephon. Nevertheless, for the ABTS method, when AVG was applied at 0.125 g L^{-1}, the reduction in TAA was even greater when compared to the fruit treated with split-doses of AVG (Table 8). Ozturk et al. (2013) also observed lower TAA in 'Braeburn' apples treated with AVG. The antioxidant activity from the phenolic compounds is a result of, mainly, their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen suppressors (Smanalieva et al., 2020).

There was an interaction between the pre-harvest treatments and harvest dates for the contents of chlorogenic acid, floridizine, epicatechin, and procyanidin B1 (Table 9). In the fruit from the first harvest, the concentration of chlorogenic acid was lower in those which received the pre-harvest application of AVG, regardless of form [(single-dose (0.125 g L^{-1}), split-doses (0.0625 g L^{-1} + 0.0625 g L^{-1}), or half-dose (0.0625 g L^{-1})], than in the fruit from the control treatment. The greatest reduction, however, occurred in the fruit treated with AVG at 0.0625 g L^{-1} 20 DBEH. In the second harvest, only the fruit that were treated with AVG at 0.0625 g L^{-1} 20 DBEH showed a reduction in the content of chlorogenic acid compared to the control treatment. The late-harvested fruit, in relation to the commercial harvest, showed a reduction in the concentration of chlorogenic acid with the control and the pre-harvest treatments (AVG at 0.0625 g L^{-1} 20 DBEH), while with the other forms of application of AVG there was no change in the content of chlorogenic acid with the delay in the harvest.

In the first harvest, any form of AVG application reduced the concentration of floridizine when compared to the control. In the second harvest, lower values of floridizine were obtained from the fruit which were treated with AVG at 0.0625 g L^{-1} 20 DBEH, if compared to the ones that received split-doses of AVG. However, none of the AVG treatments showed differences in relation to the control treatment. Compared to the commercial harvest, fruit from the late harvest showed a reduction in the concentration of floridizine in the control treatment, but not in the treatments with AVG, regardless of dose and forms of application (Table 9).

The concentration of epicatechin, in the first harvest, regardless of the forms of AVG application, was reduced in relation to the control treatment. In the second harvest, lower values of epicatechin were obtained from the fruit treated with AVG at 0.0625 g L^{-1} 20 DBEH, followed by the ones treated with AVG at 0.125 g L^{-1} 30 DBEH and the control, whereas the highest values were found in the fruit treated with split-doses of AVG. Concerning the commercial harvest, the late-harvested fruit treated with any of the treatments presented a reduction in the concentration of epicatechin (Table 9).

In the first harvest, the lowest concentration of procyanidin B1 was obtained in the fruit treated with AVG at 0.0625 g L^{-1} 20 DBEH. The same treatment, however, in the second harvest, showed the highest concentration of procyanidin B1, if compared to the fruit that received application of 0.125 g L^{-1} of AVG and the control treatment. Application of split-doses of AVG presented intermediate values, similar to the other treatments. In comparison to the commercial harvest, late-harvested fruit showed a reduction in the concentration of procyanidin B1 in the control and AVG (0.125 g L^{-1} applied 30 DBEH) treatments. The fruit treated with split-doses of AVG did not show differences in the concentration of procyanidin B1 between harvests. On the other hand, with the pre-harvest treatment of AVG at 0.0625 g L^{-1} 20 DBEH, there was an increase in the concentration of procyanidin B1 in the late-harvested fruit (Table 9).

Lower content of chlorogenic acid, floridizine, epicatechin, and procyanidin B1 in apples treated with AVG may be related to the inhibition of ethylene synthesis, while the lower activation of the phenylpropanoid metabolism in the fruit may be because of the reduction in activity of the PAL enzyme, which regulates the synthesis of all phenolic compounds (Ozturk et al., 2015). Thus, AVG can affect individual classes of phenolic compounds, reducing the concentrations of polyphenols in apples treated with AVG. AVG, however, for delaying senescence processes by decreasing ethylene production, can also reduce the use of some bioactive compounds during storage for the removal of reactive oxygen species (ROS) originating from cellular metabolism.

In late-harvested fruit, a reduction in the content of TPC and TAA, in the fruit flesh and skin, was seen in relation to the fruit of the commercial harvest (Tables 7 and 8). The reduction in the content of TPC

Table 7
Assessment of 'Baigent' apple flesh for total phenolic compounds (TPC; g EAG kg^{-1}) and total antioxidant activity [TAA; using the DPPH and ABTS methods (mol TEAC kg^{-1})] after 8 months of storage in controlled atmosphere (CA; 1.0 kPa O_2 + 2.0 kPa CO_2/0.5°C/92 % RH) plus 7 days in ambient conditions (20±1°C and 65±5 % RH).

Treatments	Commercial harvest Feb 5	Late harvest Feb 19	Mean
	TPC		
Control	2.0	1.6	1.8[ns]
AVG 0.125 g L^{-1} (30 DBEH*)	1.5	1.2	1.4
AVG 0.125 g L^{-1} (30 DBEH) + ethephon 0.120 g L^{-1} (7 DBEH)	1.8	1.8	1.8
AVG 0.0625 g L^{-1} (30 DBEH) + AVG 0.0625 g L^{-1} (20 DBEH)	1.7	1.6	1.7
AVG 0.0625 g L^{-1} (30 DBEH) + AVG 0.0625 g L^{-1} (20 DBEH) + ethephon 0.120 g L^{-1} (7 DBEH)	1.9	1.5	1.7
AVG 0.0625 g L^{-1} (20 DBEH)	1.6	1.5	1.6
Mean	1.8 a**	1.5 b	
CV (%)	10.7		
	TAA/DPPH		
Control	2.7	2.0	2.3[ns]
AVG 0.125 g L^{-1} (30 DBEH)	2.5	2.0	2.3
AVG 0.125 g L^{-1} (30 DBEH) + ethephon 0.120 g L^{-1} (7 DBEH)	2.3	2.2	2.3
AVG 0.0625 g L^{-1} (30 DBEH) + AVG 0.0625 g L^{-1} (20 DBEH)	2.3	2.0	2.2
AVG 0.0625 g L^{-1} (30 DBEH) + AVG 0.0625 g L^{-1} (20 DBEH) + ethephon 0.120 g L^{-1} (7 DBEH)	2.1	2.0	2.1
AVG 0.0625 g L^{-1} (20 DBEH)	2.4	1.9	2.2
Mean	2.4 a	2.0 b	
CV (%)	10.7		
	TAA/ABTS		
Control	2.5	2.1	2.3[ns]
AVG 0.125 g L^{-1} (30 DBEH)	2.9	1.8	2.4
AVG 0.125 g L^{-1} (30 DBEH) + ethephon 0.120 g L^{-1} (7 DBEH)	2.3	2.4	2.4
AVG 0.0625 g L^{-1} (30 DBEH) + AVG 0.0625 g L^{-1} (20 DBEH)	2.6	1.8	2.2
AVG 0.0625 g L^{-1} (30 DBEH) + AVG 0.0625 g L^{-1} (20 DBEH) + ethephon 0.120 g L^{-1} (7 DBEH)	2.5	2.3	2.4
AVG 0.0625 g L^{-1} (20 DBEH)	2.6	2.0	2.3
Mean	2.6 a	2.1 b	
CV (%)	13.6		

* DBEH: days before the expected harvest date of control treatment. **Means not followed by the same letter, uppercase in vertical and lowercase in horizontal, differ from each other by the Tukey test ($p < 0.05$). [ns]: non-significant difference.

Table 8
Evaluation of the skin of 'Baigent' apples for the total phenolic compounds (TPC; g EAG kg^{-1}) and total antioxidant activity [TAA; using the DPPH and ABTS methods (mol TEAC kg^{-1})] after 8 months of storage in controlled atmosphere (CA; 1.0 kPa O_2 + 2.0 kPa CO_2/0.5°C/92 % RH) plus 7 days in ambient conditions (20±1°C and 65±5 % RH).

Treatments	Commercial harvest Feb 5	Late harvest Feb 19	Mean
	TPC		
Control	26.5	23.8	25.1 A**
AVG 0.125 g L^{-1} (30 DBEH*)	22.4	18.3	20.4 B
AVG 0.125 g L^{-1} (30 DBEH) + ethephon 0.120 g L^{-1} (7 DBEH)	24.1	20.6	22.4 AB
AVG 0.0625 g L^{-1} (30 DBEH) + AVG 0.0625 g L^{-1} (20 DBEH)	23.1	22.0	22.6 AB
AVG 0.0625 g L^{-1} (30 DBEH) + AVG 0.0625 g L^{-1} (20 DBEH) + ethephon 0.120 g L^{-1} (7 DBEH)	22.6	20.1	21.4 AB
AVG 0.0625 g L^{-1} (20 DBEH)	20.6	21.5	21.1 AB
Mean	23.2 a	21.1 b	
CV (%)	10.8		
	TAA/DPPH		
Control	61.0	42.7	51.9 A
AVG 0.125 g L^{-1} (30 DBEH)	28.9	32.4	30.7 B
AVG 0.125 g L^{-1} (30 DBEH) + ethephon 0.120 g L^{-1} (7 DBEH)	40.8	33.3	37.1 B
AVG 0.0625 g L^{-1} (30 DBEH) + AVG 0.0625 g L^{-1} (20 DBEH)	44.5	33.8	39.2 B
AVG 0.0625 g L^{-1} (30 DBEH) + AVG 0.0625 g L^{-1} (20 DBEH) + ethephon 0.120 g L^{-1} (7 DBEH)	37.4	37.0	37.2 B
AVG 0.0625 g L^{-1} (20 DBEH)	41.8	35.8	38.8 B
Mean	42.4 a	35.9 b	
CV (%)	15.6		
	TAA/ABTS		
Control	56.9	46.3	51.6 A
AVG 0.125 g L^{-1} (30 DBEH)	41.7	37.3	39.5 C
AVG 0.125 g L^{-1} (30 DBEH) + ethephon 0.120 g L^{-1} (7 DBEH)	42.4	42.1	42.5 BC
AVG 0.0625 g L^{-1} (30 DBEH) + AVG 0.0625 g L^{-1} (20 DBEH)	47.0	45.5	46.3 B
AVG 0.0625 g L^{-1} (30 DBEH) + AVG 0.0625 g L^{-1} (20 DBEH) + ethephon 0.120 g L^{-1} (7 DBEH)	43.2	42.0	42.6 BC
AVG 0.0625 g L^{-1} (20 DBEH)	44.9	40.5	42.7 BC
Mean	46.0 a	42.3 b	
CV (%)	8.0		

* DBEH: days before the expected harvest date of control treatment. **Means not followed by the same letter, uppercase in vertical and lowercase in horizontal, differ from each other by the Tukey test ($p < 0.05$).

Table 9
Values of chlorogenic acid, floridizine, epicatechin, and procyanidin B1 (mg kg^{-1}) found on the skin of 'Baigent' apples after 8 months of storage in controlled atmosphere (CA; 1.0 kPa O_2 + 2.0 kPa CO_2/0.5°C/92 % RH) plus 7 days in ambient conditions (20±1°C and 65±5 % RH).

Treatments	Commercial harvest Feb 5	Late harvest Feb 19
	Chlorogenic acid	
Control	86.5 Aa**	30.8 Ab
AVG 0.125 g L^{-1} (30 DBEH*)	39.8 Ba	31.5 Aa
AVG 0.0625 g L^{-1} (30 DBEH) + AVG 0.0625 g L^{-1} (20 DBEH)	38.2 Ba	33.3 Aa
AVG 0.0625 g L^{-1} (20 DBEH)	25.5 Ca	11.3 Bb
CV (%)	14.1	
	Floridizine	
Control	27.8 Aa	5.3 ABb
AVG 0.125 g L^{-1} (30 DBEH)	7.4 Ba	5.9 ABa
AVG 0.0625 g L^{-1} (30 DBEH) + AVG 0.0625 g L^{-1} (20 DBEH)	7.1 Ba	6.8 Aa
AVG 0.0625 g L^{-1} (20 DBEH)	5.3 Ba	4.4Ba
CV (%)	18.6	
	Epicatechin	
Control	55.6 Aa	7.7 Bb
AVG 0.125 g L^{-1} (30 DBEH)	24.3 Ba	4.7 BCb
AVG 0.0625 g L^{-1} (30 DBEH) + AVG 0.0625 g L^{-1} (20 DBEH)	19.1 Ba	9.7 Ab
AVG 0.0625 g L^{-1} (20 DBEH)	4.4 Ba	2.5 Cb
CV (%)	41.1	
	Procyanidin B1	
Control	6.0 Aa	1.7 Bb
AVG 0.125 g L^{-1} (30 DBEH)	5.6 Aa	1.7 Bb
AVG 0.0625 g L^{-1} (30 DBEH) + AVG 0.0625 g L^{-1} (20 DBEH)	4.4 Aa	3.3 ABa
AVG 0.0625 g L^{-1} (20 DBEH)	1.3 Bb	3.9 Aa
CV (%)	24.2	

* DBEH: days before the expected harvest date of control treatment. **Means not followed by the same letter, uppercase in vertical and lowercase in horizontal, differ from each other by the Tukey test ($p < 0.05$).

in apples harvested late and stored in CA may be related to the increase in the production of free radicals due to the advance in ripening, favoring the consumption of phenolic compounds to eliminate free radicals and, consequently, reducing the TAA. These results indicate that, to better use the functional compounds, the apples stored in CA must be harvested in the commercial harvest. In contrast, the increase in the content of procyanidin B1 in apples from the late harvest which were treated with AVG at 0.0625 g L^{-1} 20 DBEH may be due to environmental conditions, cultural practices, nutrient contents, and stages of fruit ripening, as the chemical composition of the fruit, including the phenolic compounds, varies with them (Slatnar et al., 2014; Bahukhandi et al., 2018; Stanger et al., 2018).

In the fruit skin, although AVG, regardless of forms of application, dose, and combination or not with ethephon, caused a reduction in TAA; in the flesh, this effect was not observed. Apples are constituted of more flesh than skin, thus, the pre-harvest application of AVG does not present any great damage to their antioxidant potential as a whole fruit. However, studies are needed to elucidate the mechanism responsible for the maintenance of TPC in the flesh in apples treated with AVG, as well to determine whether there is a change in the metabolic pathway, which results in lower levels of specific phenolic compounds in the skin of apples that received application pre-harvest from AVG and stored in CA. In addition, studies that directly evaluate the effect of the anti-hail nets on the antioxidant activity of the fruits are needed.

4. Conclusions

The pre-harvest application of aminoethoxyvinylglycine (AVG) in split-dose and at a dose of 0.0625 g L^{-1} provides, after 8 months of storage in a controlled atmosphere (CA), apples with similar quality to those that received pre-harvest application of AVG at a dose of 0.125 g L^{-1}, indicating that they are alternatives for maintaining the quality of 'Baigent' apples produced under anti-hail nets and stored in a CA (1.0 kPa O_2 + 2.0 kPa CO_2 / 0.5°C / 92% RH). However, the pre-harvest application of ethephon after application of AVG increases the incidence of decay and mealiness, reducing the quality of the fruit.

Preharvest application of AVG, followed or not by application of ethephon, does not influence the content of total phenolic compounds (TPC) and the total antioxidant activity (TAA) of the flesh. In the skin, the application of AVG reduces the TAA, while only the application of AVG at a dose of 0.125 g L^{-1} reduces the TPC content. However, the application of AVG reduces the concentrations of chlorogenic acid, phloridizin, and epicatechin in 'Baigent' apples harvested at commercial harvest, grown under anti-hail nets, and stored in CA.

Ethical Statement - Studies in humans and animals.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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