

Article

Effects of Single and Split Pre-Harvest Aminoethoxyvinylglycine Applications in Bioactive Compounds and Antioxidant Activity in ‘Baigent’ Apples Under Anti-Hail Nets

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Abstract

The objective of this study was to evaluate the effects of single versus split pre-harvest applications of aminoethoxyvinylglycine (AVG) on the concentrations of bioactive compounds and antioxidant activity in ‘Baigent’ apple fruit cultivated under anti-hail nets, assessed at harvest and after cold storage. The pre-harvest application of AVG in a single dose (125 mg L^{−1}) in ‘Baigent’ apple trees reduces the total antioxidant activity and concentration of anthocyanins and the total phenolic compound and chlorogenic acid in the fruit skin, both at harvest and after cold storage and reduces, in the skin, the concentrations of epicatechin at harvest and of quercetin after the cold storage. The parceled application of AVG (62.5 mg L^{−1} + 62.5 mg L^{−1}) does not influence or present a less-pronounced negative effect on the total antioxidant activity and the contents of the total phenolic compound and anthocyanins in the fruit skin. Split AVG application can help maintain fruit quality during storage, providing a practical strategy for producers to optimize both marketable quality and nutritional value, potentially positively impacting commercial returns.

Keywords: *Malus domestica*; shading; growth regulators; cold storage; polyphenols; anthocyanins



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1. Introduction

The aminoethoxyvinylglycine (AVG) compound is a growth regulator applied to the field intending to delay the harvest and reduce fruit fall, in addition to delaying ripening and improving quality maintenance during storage due to reduced ethylene production [1,2]. In the ethylene biosynthesis route, AVG inhibits the conversion of S-adenosylmethionine (SAM) into 1-aminocyclopropane-1-carboxylic acid (ACC), catalyzed by the enzyme ACC synthase (ACS) [3]. Potential risks associated with this substance revolve mainly around residual toxicity and possible impacts on human health and the environment. According to leading regulatory agencies, such as the United States Environmental Protection Agency (EPA) and European agencies, AVG is considered safe for agricultural use when applied strictly according to manufacturer instructions and in line with national and international regulations. Due to its low toxicity, risks to human health are minimal, especially when pre-harvest intervals and recommended doses are observed.

AVG residues in food remain well below established maximum residue limits, posing no risk to consumers. However, misuse—such as exceeding recommended doses or applying outside of the specified period—could pose environmental and health risks, highlighting the importance of applicator training and strict adherence to official guidelines.

The application of AVG at the recommended dose and time [125 mg L^{-1} , applied 30 days before the estimated commercial harvest date (DBEHD)], causes a reduction in the red coloration in apple skin [4,5]. It was demonstrated in ‘Honeycrisp’ and ‘Gala’ apples that, despite delaying fruit ripening, AVG had a negative impact on the development of the red color [1,6]. For commercial purposes, at least 50% of the apple skin must be covered with red pigmentation to meet market acceptance standards [6]. Furthermore, AVG can alter the constitution of bioactive compounds, as well as the antioxidant potential of fruits [7].

The apple (*Malus domestica*) represents an important source of vitamin C, flavonoids, and phenolic compounds [8]. These compounds present benefits to human health through the prevention of several diseases [9] due to their antioxidant potential [10]; compounds are the most important contributors to total antioxidant activity in apples [11,12] and are derived from secondary metabolism in plants, which perform essential functions in reproduction, growth, and defense mechanisms, as well as contribute to the coloring of plants, flowers, and fruits [13,14]. They have high antioxidant potential due to their ability to neutralize and sequester free radicals [10].

The main groups of phenolic compounds in apples are phenolic acids, dihydrochalcones, flavonols, flavanols (flavan-3-ols), and anthocyanin [15,16]. According to Jakobek et al. [16] and Ceymann et al. [15], phenolic acids, dihydrochalcones, and flavonols contribute, respectively, 3–30%, 1–5%, and 2–10% of the total phenolic compound content in apples. Flavan-3-ols in monomeric forms [(+)-catechin and (–)-epicatechin] and oligomeric (proanthocyanidins) are the main flavanols and contribute 55–85% of the total phenolic compound content in apples. Anthocyanins are present in red or partially red apple cultivars, and their contribution varies from 1 to 7% of the phenolic compound content. The occurrence and content of these compounds vary between cultivars [11,16,17], skin and flesh tissue [17], orchard management [18], and storage conditions [17,19].

Currently, most apple orchards in southern Brazil are cultivated under anti-hail nets, which reduce solar radiation incident on the plants and, therefore, can interfere with the quality of the fruits [20,21]. The parceled application of the recommended dose of AVG ($62.5 \text{ mg L}^{-1} + 62.5 \text{ mg L}^{-1}$, applied at 20 and 30 DBEHD) has shown positive results in the delay of ripening and maintenance of the quality of stored fruits, without causing damage to the formation of the red color of apples [17,22]. Preharvest application of aminoethoxyvinylglycine (AVG) was more effective than 1-methylcyclopropene (1-MCP) in maintaining suppressed ethylene production in ‘NY2’ apples (RubyFrost®) during extended storage periods [23]. However, studies of the effect of AVG on anthocyanin, total phenolic compound, and total antioxidant activity content in apples grown under anti-hail nets are limited. Also, no information was found on the effect of AVG applied at the recommended dose and time (125 mg L^{-1} applied at 30 DBEHD), as well as on its split ($62.5 \text{ mg L}^{-1} + 62.5 \text{ mg L}^{-1}$, applied 20 and 30 DBEHD), on the functional properties of ‘Baigent’ apples grown under anti-hail nets, at harvest and after cold storage.

The objective of this study was to evaluate the effects of single versus split preharvest applications of aminoethoxyvinylglycine (AVG) on the concentrations of bioactive compounds and antioxidant activity in ‘Baigent’ apple fruit cultivated under anti-hail nets, assessed at harvest and after cold storage.

2. Materials and Methods

2.1. Location of the Orchard

The experiment was carried out from January to December 2018 with ‘Baigent’ apples in a commercial orchard located in the municipality of Vacaria, RS, Brazil (50°42′ W; 28°33′ S; and 955 m above sea level), covered with black anti-hail nets with a mesh opening of 4 × 7 mm, 25% to 35% photosynthetic active radiation (PAR), installed in 2010. 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 [24]. According to the Köppen–Geiger’s classification, the climate is ‘Cfb’, constantly moist temperate with mild summers.

2.2. Treatments

The treatments consisted of a control (plants sprayed with water); single-dose AVG (125 mg L^{−1}, sprayed 30 DBEHD); and split-dose AVG (62.5 mg L^{−1} + 62.5 mg L^{−1}, sprayed 30 and 20 DBEHD). The source of AVG was ReTain®. The adhesive spreader used in the treatments was Break Thru (0.05% v/v). For each treatment, two harvests were performed: the first at the commercial ripening stage of the control treatment and the second 14 days later. The fruit (100) were randomly harvested from the middle third of the plant canopy. For storage, 20 fruits per experimental unit were used, without damage or defects. The choice of 20 apples per batch is standard in postharvest studies to ensure statistical power and reduce variability; this batch size is commonly supported in the literature. The applied AVG dosages (single: 125 mg L^{−1}; split: 62.5 mg L^{−1} + 62.5 mg L^{−1}) follow agronomic recommendations and previously published studies.

2.3. Storage Conditions and Analyzed Variables

The fruits were evaluated at harvest and after four months of cold storage (0.5 ± 0.2 °C and RH 92 ± 5%), followed by seven days in ambient conditions (20 ± 5 °C and RH 63 ± 2%) to simulate the marketing period. The following attributes were evaluated: total phenolic compound (mg GAE kg^{−1} FW) and total antioxidant activity (DPPH and ABTS methods; μMol trolox kg^{−1} FW) in the skin and flesh, and total anthocyanin (mg cyanidine 3-glucoside kg^{−1} FW) and chlorogenic acid, floridizine, epicatechin, and procyanidin B1 (mg kg^{−1} FW in the skin). The evaluations were analyzed as described in Stanger et al. [17]. The analysis of chlorogenic acid, floridizine, epicatechin, and procyanidin B1 were only performed in Harvest 2.

Analytical grade reagents, including 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Folin–Ciocalteu reagent, sodium acetate, and potassium persulfate, were sourced from Sigma Chemical Co. (St. Louis, MO, USA). Standard solutions for chlorogenic acid, catechin, epicatechin, procyanidins B1 and B2, quercetin 3-galactoside, and phloridzin, as well as HPLC-grade solvents—acetonitrile, acetic acid, and methanol—were also obtained from Sigma Chemical Co. Additional chemicals such as gallic acid, sodium carbonate, acetone, and ethanol were purchased from Vetec (Rio de Janeiro, Brazil) with analytical grade quality. Peels were carefully removed from the entire fruit surface using a sharp blade approximately 1 mm thick. Pulp samples were collected by slicing a longitudinal segment (about 1 cm thick) from the fruit’s center, excluding the endocarp and preserving the sides of the slice. Pulp was homogenized using a vertical grinder (model RI1364, Philips Walita, Varginha, Brazil), and peels were pulverized in a mortar with liquid nitrogen.

Extraction for total phenolic content (TPC) and total antioxidant activity (TAA) quantification followed the approach outlined by Rufino et al. [25], with modifications based on

Larrauri et al. [26] For this, 10.0 g of pulp and 2.5 g of peel were each mixed in a Falcon tube (Zollstr, Switzerland) with 10 mL of a 1:1 methanol/distilled water solution. These were homogenized using an Ultra-Turrax disperser (model D-91126, Heidolph, Schwabach, Germany) and allowed to stand for 60 min at room temperature. Following this, samples were centrifuged in an Eppendorf 5810R centrifuge (Hamburg, Germany) at 12,880 g for 20 min at 4 °C. The supernatant was decanted into a 25 mL volumetric flask, and a second extraction was performed by treating the residue with 10 mL of 70:30 acetone/distilled water. The mixture was homogenized and left to stand for another 60 min at 20 °C before another round of centrifugation under the same conditions. The second supernatant was combined with the first, and the volume was topped up to 25 mL with distilled water.

To determine TPC, a modified version of the spectrophotometric Folin–Ciocalteu method, described by Roesler et al. [27], was used. Each assay included 2.5 mL of a Folin–Ciocalteu/distilled water mixture (25:75, *v/v*) added to 0.5 mL of appropriately diluted hydroalcoholic extract in triplicate. The mixture was vortexed and allowed to react for 3 min at 20 °C. Then, 2.0 mL of sodium carbonate solution (10% *w/v*) was added, followed by another mixing. Samples were left in the dark for one hour before measuring absorbance at 765 nm using a BEL2000-UV spectrophotometer (Bel Photonics, Piracicaba, Brazil). TPC was quantified via the calibration curve with gallic acid, and results were presented as mg gallic acid equivalents per 100 g of fresh mass (mg GAE 100 g^{−1} FM).

TAA was evaluated using both ABTS and DPPH assays. For the ABTS method, radical cations were produced by incubating an ABTS stock solution (7 mM) with potassium persulfate (140 mM) in the dark for 16 h at 20 °C. Prior to analysis, radicals were adjusted with ethanol to reach an absorbance of 0.70 ± 0.05 at 734 nm. Three dilutions of extract were tested in triplicate by adding 30 µL of extract to 3.0 mL of ABTS solution, followed by vortexing; absorbance was read at 734 nm after 6 min. Trolox standards were used for calibration, and results were reported as a Trolox equivalent antioxidant capacity (TEAC) in µmol TEAC g^{−1} FM.

The DPPH test involved preparing a 0.06 mM DPPH solution in methanol on the day of testing. Then, 0.1 mL of hydroalcoholic extract was mixed with 3.9 mL of DPPH reagent in triplicate, followed by vortexing. Absorbance was measured at 515 nm after 30 min. Trolox standards generated the calibration curve, and antioxidant capacity was expressed as µmol TEAC per 100 g of fresh mass.

Total anthocyanins (TANs) were quantified using a method adapted from Fuleki and Francis [28]. For this, 5.0 g of apple peel was mixed with 15 mL of 95:5 (*v/v*) ethanol/distilled water and acidified with ethanol/hydrochloric acid (1.5 N HCl, 85:15, and *v/v*). Samples were homogenized (SilentCrusher M, Heidolph), maintained at 4 °C for 24 h, and centrifuged at 12,880 g for 20 min at 4 °C (Eppendorf 5810R). From the supernatant, 2 mL was transferred to a 50 mL volumetric flask, filled to volume with extraction solvent, and absorbance was measured at 535 nm with a BEL2000-UV spectrophotometer. Results were expressed as mg cyanidin 3-glucoside per 100 g of fresh mass.

Phenolic compound profiling (IPC) was conducted via HPLC, following Tsao et al. [29]. Sample preparation paralleled the procedures for TPC and TAA: extracted samples (1:1 *w/v*) in methanol/ultrapure water (70:30, *v/v*) were homogenized, vacuum filtered, and further filtered through 0.45 µm syringe filters (Kasvi, Curitiba, Brazil). Samples were stored at −20 °C until analysis. HPLC analysis was performed using a Shimadzu system (Tokyo, Japan) equipped with an SCL-10AVP controller, FCV-10AL VP gradient mixer, LC-10ADVP pump, SIL 10-ADVP auto-injector, and SPD-10A VP UV detector, utilizing a CLASS VP (version 6.14) software and a C18 column (250 × 4.6 mm, 5 µm, Restek, PA, USA). The mobile phase used was acetic acid/ultrapure water (6:94, *v/v*) with 2 mM sodium acetate buffer, pH 2.55 (solvent A), and acetonitrile (solvent B), with a stepwise gradient elution

over 80 min at a flow rate of 1.0 mL/min. Detection occurred at 280 nm and injections were 20 µL. Phenolic compounds were identified by matching retention times with standards for procyanidins B1 (10.9 min), catechin (17.4 min), procyanidin B2 (22.0 min), chlorogenic acid (22.7 min), epicatechin (35.2 min), quercetin 3-galactoside (52.6 min), and phloridzin (67.0 min). A spectral library, including retention times and UV spectra at 280 nm, was developed for compound confirmation. Quantification used external calibration curves (0–100 mg g^{−1}) with standards in methanol, and standard solutions were run at both the start and end of each batch to ensure accuracy, with recovery rates between 97% and 110%. All analyses were performed in duplicate.

2.4. Experimental Design and Statistical Analysis

The experimental design used was in random blocks, according to a 3 × 2 factorial (three treatments and two harvests) with four replicates. Each repetition is composed of 20 fruits. The data were submitted to an analysis of variance and means of treatment compared by the Tukey test ($p < 0.05$). The total phenolic compound variable was submitted to a Pearson's correlation analysis ($p < 0.001$) with total antioxidant activity. The statistical analyses were performed using the SAS program (SAS Institute, 2002, version 9.0).

3. Results and Discussion

3.1. When Harvesting Fruit

A positive and highly significant correlation ($p < 0.001$) was observed between total phenolic compound content and total antioxidant activity (by the ABTS and DPPH methods) in the skin and flesh and between the total phenolic compound content and the anthocyanin content in the skin in both harvests (Table 1). Stanger et al. [17,19] and Soethe et al. [30] also observed a positive linear correlation between the total phenolic compound and total antioxidant activity in apples at harvest and after storage. This indicates that phenolic compounds are important phytochemicals that contribute to antioxidant activity in apples. The structure, particularly the number and position of the hydroxyl groups and the nature of the replacements of the aromatic rings, are the main characteristics of the phenolic compounds responsible for the antioxidant activity [31,32].

Table 1. Pearson correlation coefficients (r) between total phenolic compounds (TPC) and total antioxidant activity, quantified by ABTS and DPPH methods, and anthocyanins (ANT), in the ‘Baigent’ apple skin and flesh portions, evaluated in the commercial harvest, at two harvest dates.

Correlation	Harvest 1		Harvest 2	
	Skin	Flesh	Skin	Flesh
TPC × DPPH	0.99 ***	0.99 ***	0.99 ***	0.95 ***
TPC × ABTS	0.99 ***	0.81 ***	0.82 ***	0.96 ***
TPC × ANT	0.99 ***	-	0.90 ***	-

*** significant ($p < 0.001$).

No difference was observed between the treatments for the total phenolic compound and total antioxidant activity content of the flesh in the two harvests evaluated (Figure 1). Ozkan et al. [8], working with ‘Braeburn’ apples, and Awad and Jager [32], working with ‘Jonagold’ apples, also did not notice any change in the total phenolic compound content of the flesh with a pre-harvest application of AVG.

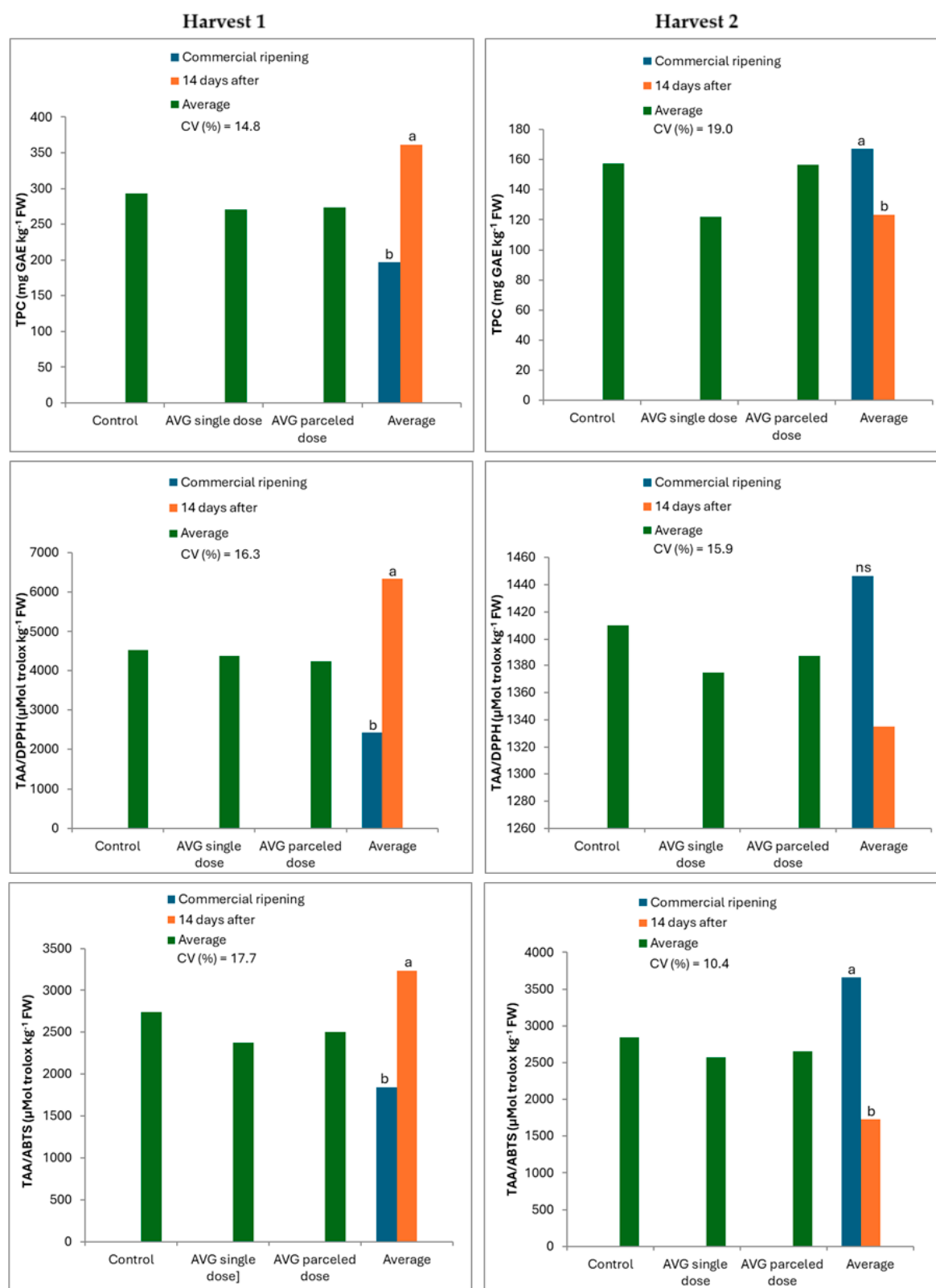


Figure 1. Total phenolic compound contents (TPC; mg GAE kg⁻¹ FW) and total antioxidant activity (TAA; DPPH and ABTS methods; and μMol trolox kg⁻¹ FW) in ‘Baigent’ apple flesh, in response to pre-harvest application of aminoethoxyvinylglycine (AVG) (AVG single dose (125 mg L⁻¹/30 DBEHD—days before the estimated harvest date) and AVG parceled dose (62.5 mg L⁻¹ + 62.5 mg L⁻¹/30 and 20 DBEHD)) and two harvest dates. Treatment averages, comparing harvest dates, not followed by the same lower-case letter, differ by Tukey test ($p < 0.05$). ns: non-significant difference between treatments for average data of the two harvest dates.

There was no significant interaction between treatments and harvest dates for the total phenolic compound, total antioxidant activity (ABTS and DPPH methods), and anthocyanin quantified in the fruit skin (Figure 2). Pre-harvest application of AVG in a single dose (125 mg L^{-1}) provided fruits with a lower total phenolic compound content in the skin compared to the control treatment fruits in the two harvests evaluated. The parceled application of AVG ($62.5 \text{ mg L}^{-1} + 62.5 \text{ mg L}^{-1}$) did not differ from the control for the total phenolic compound content in the skin in Harvest 1 but reduced in Harvest 2. The reduction in total phenolic compound content in fruits treated with AVG was also observed by Ozturk et al. [33] in plums, Ozturk and Kucuker [34] and Yildiz et al. [35] in sweet cherries, and Ozturk et al. [7] in 'Braeburn' apples. According to Ozturk et al. [33], the decrease in the content of bioactive compounds by AVG may be due to inhibition of the synthesis of ethylene, because the stimulus to the production of ethylene has a consequence on the activation of the metabolism of phenylpropanoids in vegetables, due to the increase in the activity of the enzyme phenylalanine ammonia-lyase (PAL), which regulates the synthesis of phenolic compounds [36]. Khan et al. [37] indicate that the decrease in total phenolic compound content in fruits treated with AVG is related to the inhibition of the normal production of free radicals during the respiratory process and, consequently, the non-activation of secondary metabolism.

In Harvest 1, fruits of the plants that received the pre-harvest application of AVG in a single dose presented a lower total antioxidant activity content in the skin (ABTS and DPPH methods) than the control, while the application of parceled AVG did not differ from the control (Figure 2). In Harvest 2, any form of application of AVG provided a reduction in total antioxidant activity by the DPPH method, while total antioxidant activity by the ABTS method's reduction concerned the control only with the pre-harvest application of AVG in a single dose. Ozturk et al. [7] and Ceymann et al. [15] also observed a reduction in total antioxidant activity on 'Braeburn' apple skin that received a single dose application of AVG ($100; 300; 500 \text{ mg L}^{-1}$), sprayed four weeks before the estimated harvest date. Lower total antioxidant activity in apples that received a pre-harvest application of AVG in a single dose reflects the lower total phenolic compound content, considering the positive and highly significant correlation between the total phenolic compound and total antioxidant activity (Table 1).

In Harvest 1, there was a reduction in anthocyanin content in fruits that received the pre-harvest application of AVG in a single dose, while apples that received the pre-harvest application of AVG in parcel showed no change in anthocyanin content compared to the control treatment fruits (Figure 2). In Harvest 2, any form of application of AVG showed a reduction in anthocyanin content compared to the fruits of the control treatment; however, the pre-harvest application of AVG in a single dose showed an even greater reduction when compared to the fruits that received a parceled application of AVG. The reduction in anthocyanin content in apples that received an application of AVG [single dose ($100; 300; 500 \text{ mg L}^{-1}$), sprayed four weeks before the estimated harvest date] was also reported by Ozturk et al. [7] in 'Braeburn' apples. According to Awad et al. [38] and Li et al. [39], ethylene triggers the expression of genes from the biosynthesis of anthocyanin, because this phytohormone leads to the activation of secondary metabolism as a result of the increased activity of PAL, while AVG inhibits or delays its expression due to a reduction in the rate of ethylene production and, consequently, a lower concentration of anthocyanin in the fruit. Additionally, the use of anti-hail nets, by virtue of their shading effect, reduces the incident sunlight and can negatively impact the accumulation of anthocyanin on the epidermis (responsible for the red skin color) [20,21,40], presenting a negative synergistic effect with AVG.

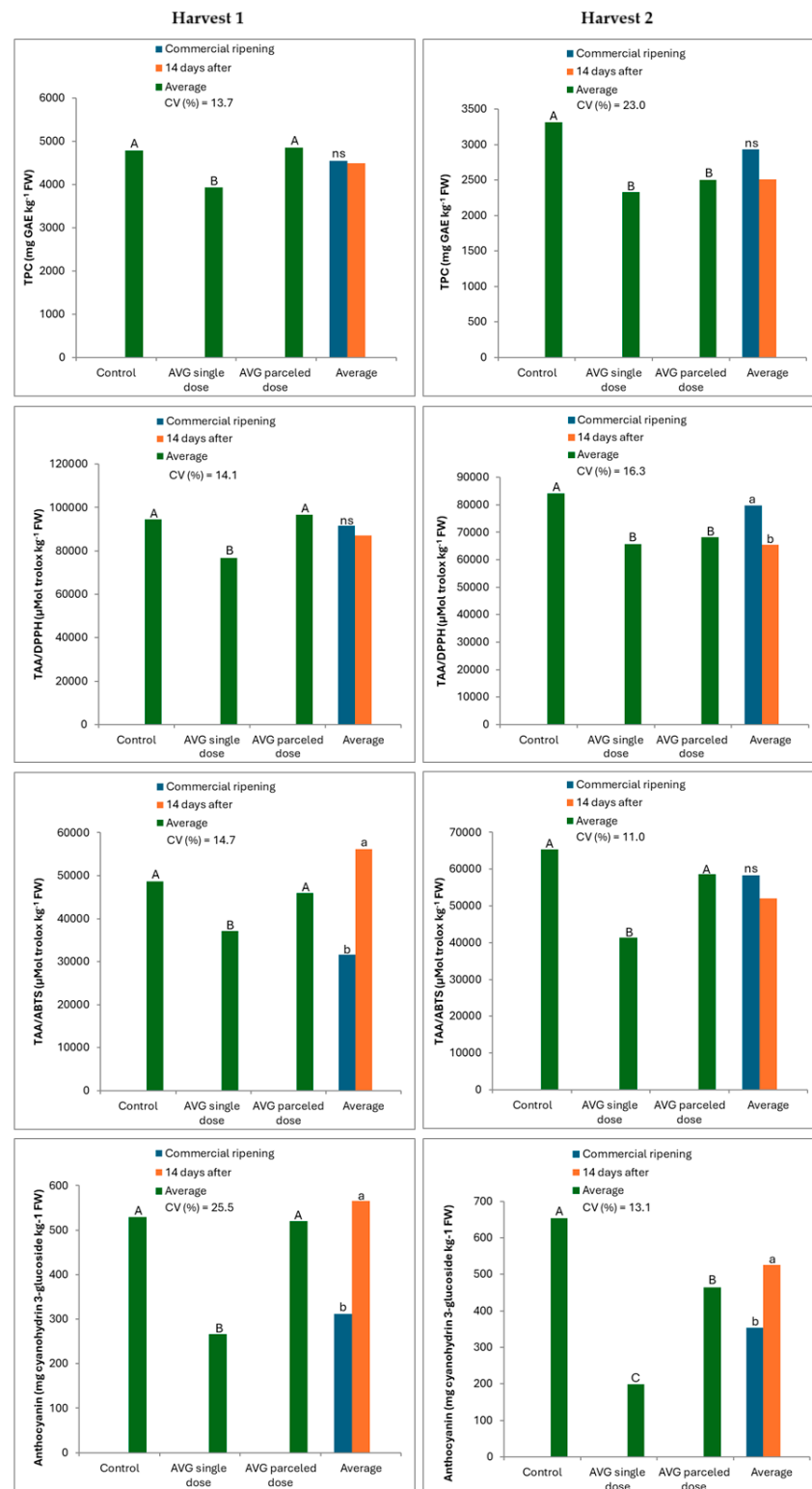


Figure 2. Total phenolic compound contents (TPC; mg GAE kg⁻¹ FW), total antioxidant activity (TAA; DPPH and ABTS methods; and μMol trolox kg⁻¹ FW), and anthocyanin content (mg cyanohydrin 3-glucoside kg⁻¹ FW) in ‘Baigent’ apple skin, in response to pre-harvest application of aminoethoxyvinylglycine (AVG) (AVG single dose (125 mg L⁻¹/30 DBEHD—before the estimated harvest date) and AVG parceled dose (62.5 mg L⁻¹ + 62.5 mg L⁻¹/30 and 20 DBEHD)) and two harvest dates. Treatment means that, when comparing harvest dates, are not followed by the same lowercase letter, and treatment means with or without AVG application that are not followed by the same uppercase letter, differ according to Tukey’s test ($p < 0.05$). ns: non-significant difference between treatments, based on the average of the two harvest dates.

The application of AVG in a single dose reduced the concentration of chlorogenic acid and epicatechin in the skin, while the parceled application of AVG did not influence its concentration compared to the fruits of the control treatment (Figure 3). The lower content of chlorogenic acid and epicatechin in apples treated with AVG in a single dose 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 [33]. Thus, AVG can affect individual classes of phenolic compounds, reducing the concentrations of polyphenols in apples treated with AVG. On the other hand, any form of pre-harvest application of AVG has increased the content of floridizine and catechin in the skin, compared to the fruits of the control treatment. The quercetin content in the skin increased with the pre-harvest application of AVG in a single dose compared to the control treatment. In contrast, the parceled application of AVG did not differ among the other treatments. Kucuker et al. [41], when evaluating the whole fruit, also observed a lower chlorogenic acid content in plums that received a pre-harvest application of AVG. Ozturk et al. [33] observed a reduction in the content of chlorogenic acid and quercetin in plums, evaluated throughout the fruit treated with the highest dose of AVG (200 mg L^{-1}) but not with the lowest dose (100 mg L^{-1}). Therefore, the effect of AVG can be dose-dependent. In ‘Huangguan’ pears, AVG application decreased the malondialdehyde (MDA) content and polyphenol oxidase (PPO) activity, delayed the peak of chlorogenic acid (CGA) content in the core tissue, and significantly inhibited the expression of genes such as ACC synthase, ACC oxidase, ethylene receptors, ethylene response factor, phenylalanine ammonia lyase, cinnamate 4-hydroxylase, 4-hydroxycinnamoyl-CoA ligase, hydroxycinnamoyl-CoA shikimate hydroxycinnamoyl transferase, and polyphenol oxidase, as well as phospholipase D and lipoxygenase. [42]. In ‘Cripps Pink’ apples skin tissues, different from the present work, the application of AVG did not influence the content of chlorogenic acid, epicatechin, floridizine, catechin, and quercetin [43]. The differences observed between the different works and the present study may be related to the species and cultivars used in the study. The chemical composition of fruits, including phenolic compounds, varies according to the cultivar, environmental conditions, cultural practices, nutrient contents, and fruit ripening stage [17,19,44]. In addition, the methods of analysis used and the type of tissue sampled (skin, flesh, or whole fruit) can also produce, among the various research works, different results for phenolic compounds [17,45].

Individual phenolic compounds have a premium contribution in the antioxidant activity [12,46], the phenolic acids being the main acids responsible for the antioxidant activity in apples, with an emphasis on chlorogenic acid [47]. However, other studies consider glycosylated quercetin as one of the phenolic compounds, which contribute most to the antioxidant activity in apples, because it has structural advantages over other molecules [48,49], being one of the most effective compounds in all antioxidative parameters [13]. In the present study, the total antioxidant activity was superior in fruits in the control treatment (Figure 2), which had the lowest quercetin content and the highest chlorogenic acid content (Figure 3).

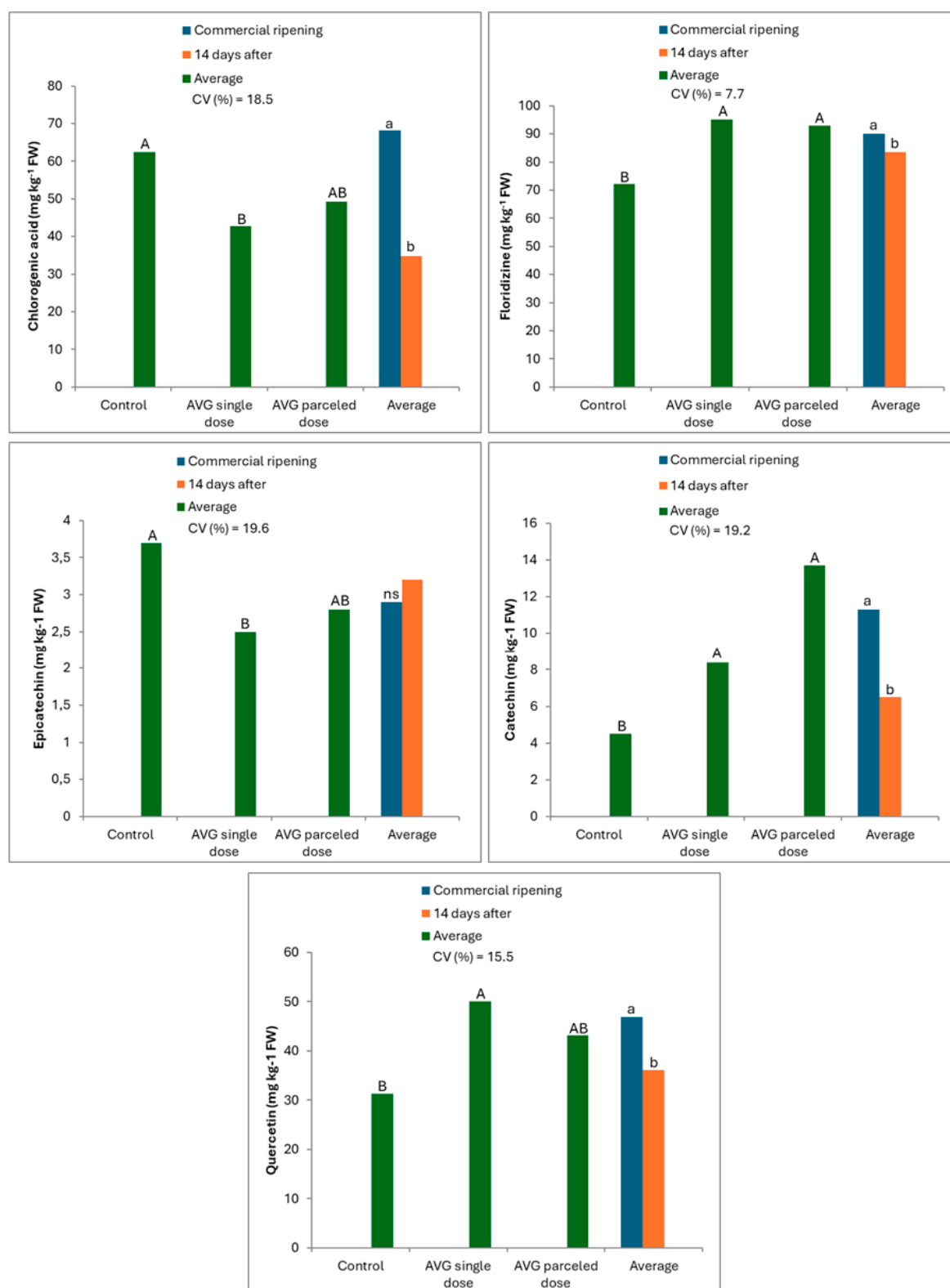


Figure 3. Chlorogenic acid, floridizine, epicatechin, catechin, and quercetin (mg kg⁻¹ FW) levels in ‘Baigent’ apple skin, in response to pre-harvest application of aminoethoxyvinylglycine (AVG) (AVG single dose (125 mg L⁻¹/30 DBEHD—before the estimated harvest date) and AVG parceled dose (62.5 mg L⁻¹ + 62.5 mg L⁻¹/30 and 20 DBEHD)) and two harvest dates in Harvest 2. Treatment means that, when comparing harvest dates, are not followed by the same lowercase letter, and treatment means with or without AVG application that are not followed by the same uppercase letter, differ according to Tukey’s test ($p < 0.05$). ns: non-significant difference between treatments, based on the average of the two harvest dates.

With the harvest delay, there was an increase in the total phenolic compound and total antioxidant activity values (DPPH and ABTS methods) in the flesh for Harvest 1. In Harvest 2, there was a reduction in the total phenolic compound and total antioxidant activity content by the ABTS method in the flesh but not in the total antioxidant activity by the DPPH method with a delay in harvesting (Figure 1). In the skin, no change in the total phenolic compound content was observed with the harvest delay in the two harvests evaluated. In Harvest 1, by the DPPH method, no difference was observed in the total antioxidant activity in the skin between the two harvests, while by the ABTS method, there was an increase in the total antioxidant activity in the shell with the delay in harvesting. In Harvest 2, there was a reduction in total antioxidant activity by the DPPH method in the skin with the harvest delay but not the total antioxidant activity by the ABTS method (Figure 2). However, the occurrence and content of phenolic compounds and antioxidant capacity vary between cultivars [17], orchard management [18], sun exposure, and stage of ripening [50].

The anthocyanin content increased with the delay of harvest in the two harvests evaluated (Figure 2), indicating an increase in the red skin color of the fruits, as also observed by Whale et al. [43] and Li et al. [51]. However, with the harvest delay, there was a reduction in the content of chlorogenic acid, floridizine, catechin, and quercetin but not of epicatechin (Figure 3). These different phenolic compounds are intermediate in the biosynthesis route of anthocyanin, and their reduction may be related to the increase in anthocyanin content.

3.2. After Cold Storage (CS)

In the flesh, after four months of cold storage, no difference was observed between pre-harvest treatments for the total phenolic compound and total antioxidant activity values (DPPH and ABTS methods) in the two harvests evaluated (Figure 4). Kucuker et al. [45] also did not observe the effect of AVG on these variables in the plum flesh after cold storage. These results agree with the findings of Aglar et al. [52], who reported that the application of AVG had no significant effect on the total antioxidant activity or the total phenolic compound content in jujube fruits after cold storage. According to Rotili et al. [53], total antioxidant activity in fruits results from the action of a variety of compounds that are degraded or synthesized during storage in response to biotic and abiotic stresses.

In the skin, during Harvest 1, treatment with AVG, regardless of the form of application, caused a reduction in the total phenolic compound content compared to the fruits of the control treatment (Figure 5). In Harvest 2, only the pre-harvest application of AVG in a single dose provided a lower total phenolic compound content compared to the control. The pre-harvest parceled application of AVG presented intermediate values not differing from the other treatments. Therefore, the pre-harvest application of AVG in a split dose showed a less-pronounced negative effect than the application of AVG in a single dose on the total phenolic compound content in the skin of fruits kept in cold storage. Karaman et al. [54] and Ozturk et al. [34] also observed a lower total phenolic compound content in plums that received a pre-harvest application of AVG after the cold storage period. The lower total phenolic compound content in ap-ples treated with AVG may be related to a reduced ethylene production rate in the fruit. According to Picoli et al. [55], the increase in the rate of ethylene production induces the activity of the enzyme PAL, which regulates the synthesis of phenolic compounds, thus AVG can reduce the content of the total phenolic compound by reducing the synthesis of ethylene.

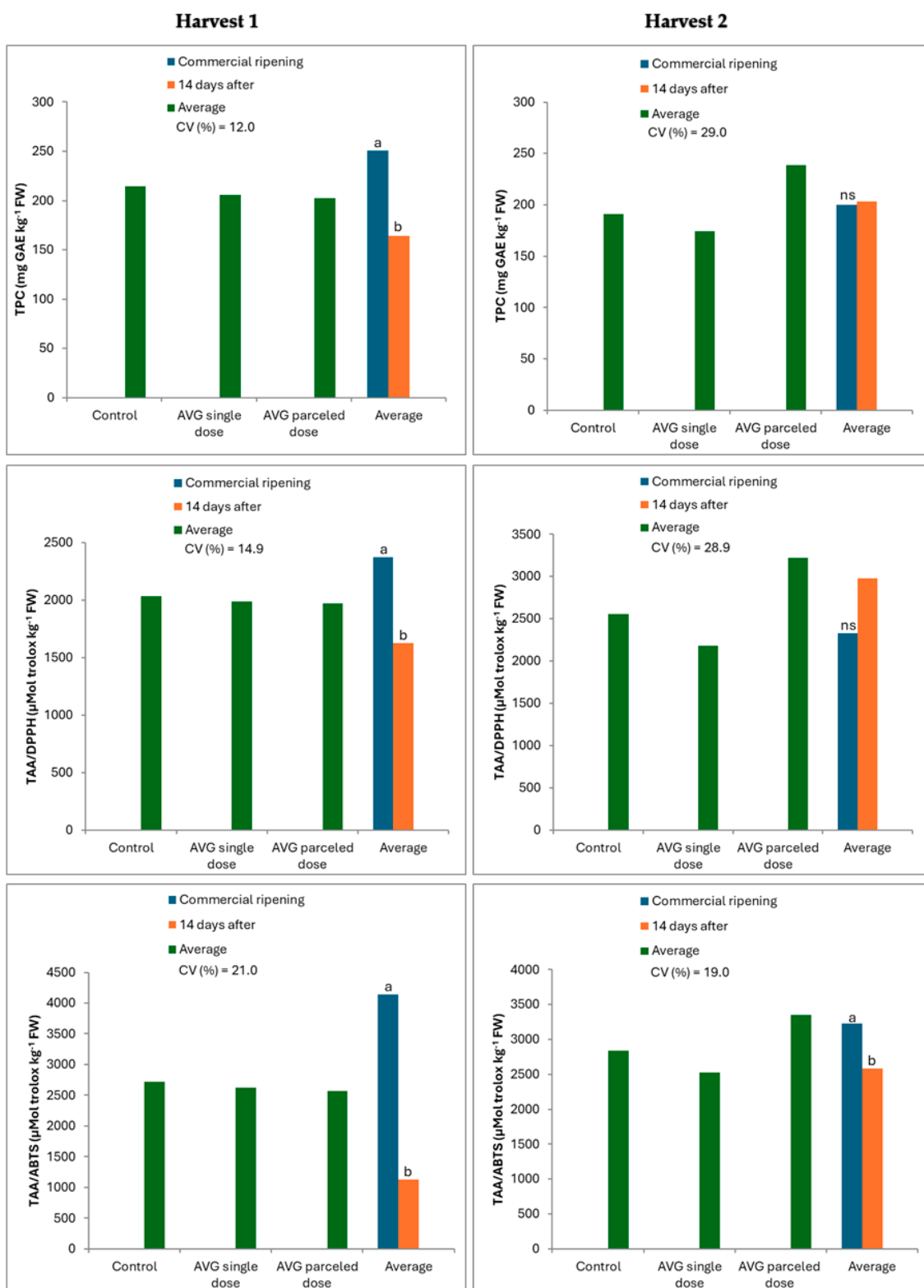


Figure 4. Total phenolic compound contents (TPC; mg GAE kg⁻¹ FW) and total antioxidant activity (TAA; DPPH and ABTS methods; and μMol trolox kg⁻¹ FW) in ‘Baigent’ apple flesh, in response to pre-harvest application of aminoethoxyvinylglycine (AVG) (AVG single dose (125 mg L⁻¹/30 DBEHD—before the estimated harvest date) and AVG parceled dose (62.5 mg L⁻¹ + 62.5 mg L⁻¹/30 and 20 DBEHD)) and two harvest dates, evaluated after four months of cold storage, followed by seven more days in ambient conditions (20 ± 1 °C and 65 ± 5% RH). Treatment averages comparing harvest dates, not followed by the same lower-case letter, differ by Tukey test ($p < 0.05$). ns: non-significant difference between treatments for average data of the two harvest dates.

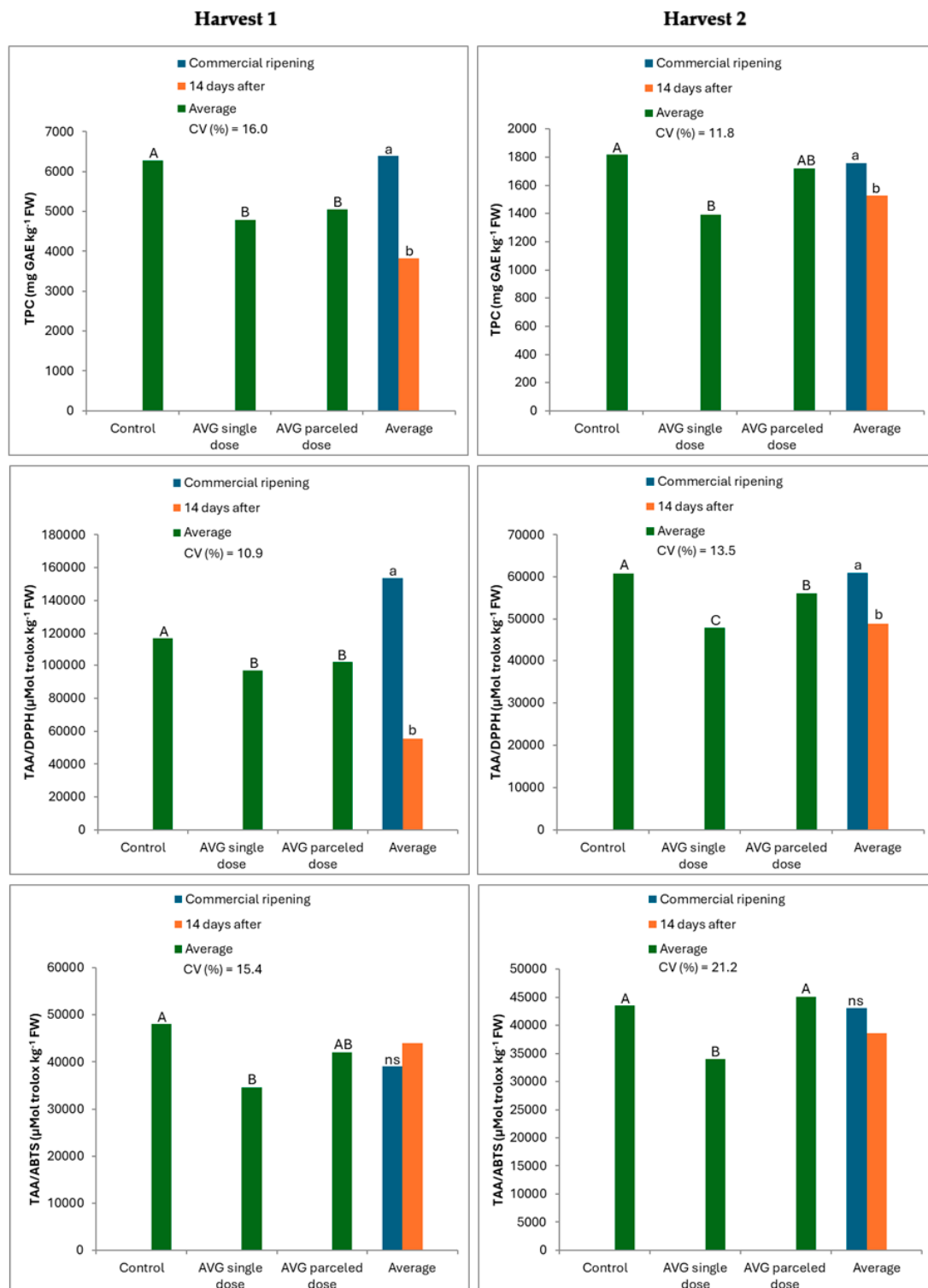


Figure 5. Total phenolic compound contents (TPC; mg GAE kg⁻¹ FW) and total antioxidant activity (TAA; DPPH and ABTS methods; and μMol trolox kg⁻¹ FW) in ‘Baigent’ apple skin, in response to pre-harvest application of aminoethoxyvinylglycine (AVG) (AVG single dose (125 mg L⁻¹/30 DBEHD—before the estimated harvest date) and AVG parceled dose (62.5 mg L⁻¹ + 62.5 mg L⁻¹/30 and 20 DBEHD)) and two harvest dates after four months of cold storage, followed by seven more days in ambient conditions (20 ± 1 °C and 65 ± 5% RH). Treatment means that, when comparing harvest dates, are not followed by the same lowercase letter, and treatment means with or without AVG application that are not followed by the same uppercase letter, differ according to Tukey’s test ($p < 0.05$). ns: non-significant difference between treatments, based on the average of the two harvest dates.

In the skin, for both seasons, any form of pre-harvest application of AVG reduced the total antioxidant activity quantified through the DPPH method, compared to the fruits of the control treatment (Figure 5). However, in Harvest 2, the reduction in total antioxidant activity quantified by the DPPH method in single-dose AVG-treated apples was greater than in apples that received a parceled application of AVG. For the total antioxidant activity quantified by the ABTS method, only the pre-harvest application of single-dose AVG reduced the total antioxidant activity relative to the control in both seasons. These results indicate that the parceled application of AVG has a less-intense negative impact than single-dose AVG after cold storage, presenting similar or closer values to the fruits of the control treatment. Karaman et al. [54] also observed lower total antioxidant activity in plums that received the pre-harvest application of AVG after the cold storage period than those of the control treatment.

There was interaction between the pre-harvest treatment factors and harvest dates for the chlorogenic acid content (Table 2). In the commercial ripening, the pre-harvest application of AVG in a single dose and parcel showed lower chlorogenic acid content compared to the fruits of the control treatment. In the 14 days after harvest, only the application of AVG in a parceled dose showed a lower chlorogenic acid content compared to the fruits of the control treatment. Ozturk et al. [34] also observed a lower chlorogenic acid content in plums treated with AVG after the cold storage period.

Table 2. Chlorogenic acid (mg kg^{-1} FW) contents of ‘Baigent’ apple skin, in response to pre-harvest application of aminoethoxyvinylglycine (AVG) (AVG single dose ($125 \text{ mg L}^{-1}/30 \text{ DBEHD}$ —before the estimated harvest date) and AVG parceled dose ($62.5 \text{ mg L}^{-1} + 62.5 \text{ mg L}^{-1}/30$ and 20 DBEHD)) and two harvest dates, evaluated after four months of cold storage, followed by seven more days in ambient conditions ($20 \pm 1^\circ \text{C}$ and $65 \pm 5\% \text{ RH}$) for Harvest 2.

Treatments	Commercial Ripening	14 Days After	Average
Chlorogenic acid			
Control	128.6 Aa	59.2 Ab	-
AVG single dose ($125 \text{ mg L}^{-1}/30 \text{ DBEHD}^*$)	67.3 Ba	59.4 Aa	-
AVG parceled dose ($62.5 \text{ mg L}^{-1} + 62.5 \text{ mg L}^{-1}/30$ and 20 DBEHD)	35.4 Ca	34.6 Ba	-
Average	-	-	-
CV (%)	17.5		

* DBEHD: Before the estimated harvest date. Averages not followed by the same letter, upper case in the columns and lower case in the rows, differ by Tukey test ($p < 0.05$).

There was no effect of the AVG pre-harvest application on the floridizine content (Figure 6). For catechin, only the split application of AVG reduced its content compared to the fruits of the control treatment. Lower quercetin content was observed in the fruits that received an application of AVG, independent of the form. The results of this work are in accordance with those obtained in plums, where there was a reduction in the concentration of catechin and quercetin with the application of AVG after cold storage [34].

In Harvest 1, there was a reduction in the total phenolic compound and total antioxidant activity content in the flesh with the harvest delay. In Harvest 2, there was no change in the total phenolic compound content and in the total antioxidant activity by the DPPH method, while in the total antioxidant activity by the ABTS method, there was a reduction with the harvest delay (Figure 4). In the skin, a reduction in the total phenolic compound content and in the total antioxidant activity by the DPPH method was observed with the harvest delay, while no change in the total antioxidant activity by the ABTS method was observed in the two seasons evaluated (Figure 5). There was a reduction in floridizine and chlorogenic acid concentrations in the fruits of the control treatment with a harvest delay,

while in fruits with AVG applications, there was no effect of the harvest date on chlorogenic acid concentration. On the other hand, an increase in the quercetin concentration was observed, and no difference was observed for the catechin concentration with a harvest delay (Figure 6).

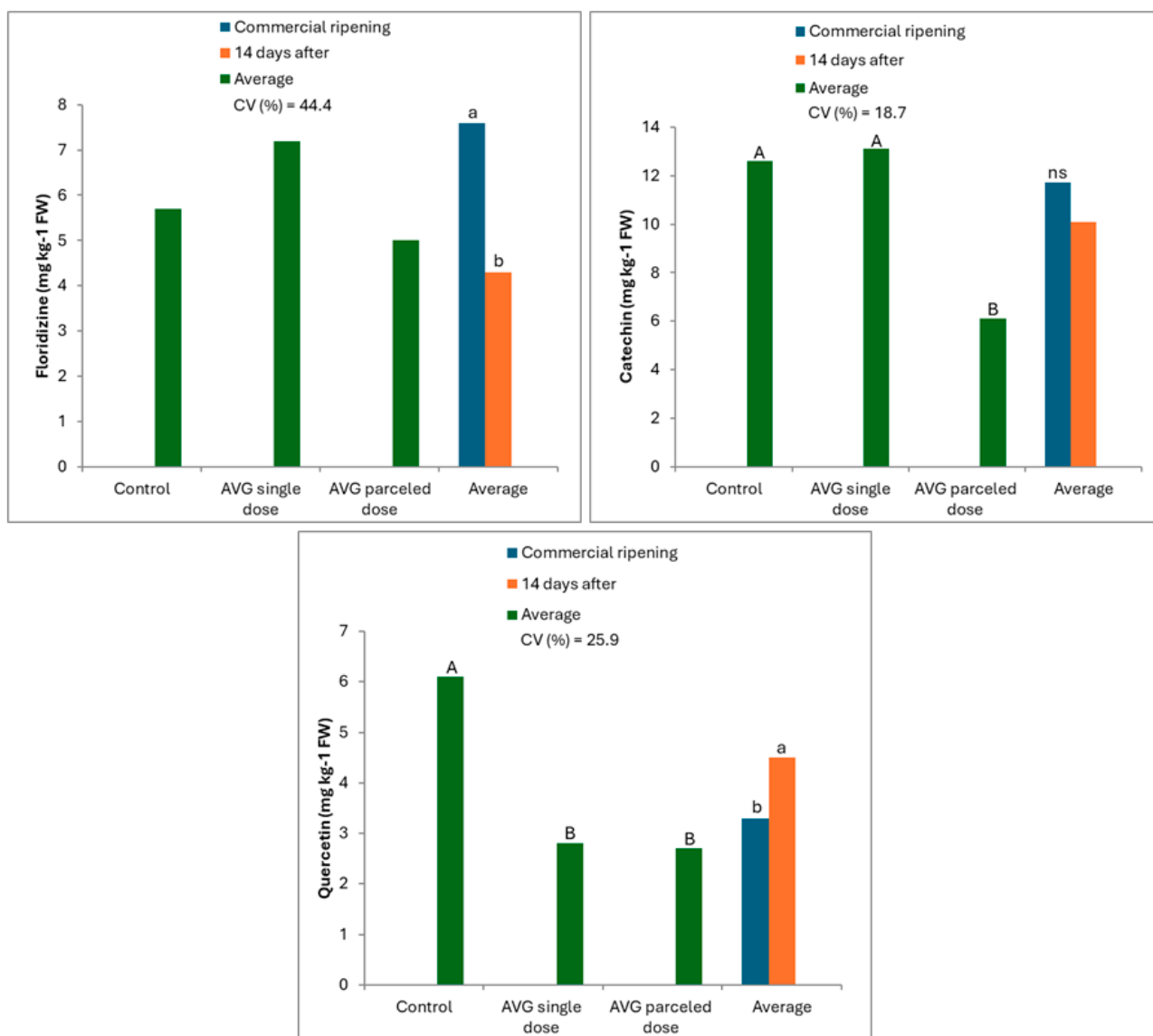


Figure 6. Floridizine, catechin, and quercetin (mg kg^{-1} FW) contents of ‘Baigent’ apple skin, in response to pre-harvest application of aminoethoxyvinylglycine (AVG) (AVG single dose ($125 \text{ mg L}^{-1}/30 \text{ DBEHD}$ —before the estimated harvest date) and AVG parceled dose ($62.5 \text{ mg L}^{-1} + 62.5 \text{ mg L}^{-1}/30$ and 20 DBEHD)) and two harvest dates, evaluated after four months of cold storage, followed by seven more days in ambient conditions ($20 \pm 1^\circ\text{C}$ and $65 \pm 5\% \text{ RH}$) for Harvest 2. Treatment means that, when comparing harvest dates, are not followed by the same lowercase letter, and treatment means with or without AVG application that are not followed by the same uppercase letter, differ according to Tukey’s test ($p < 0.05$). ns: non-significant difference between treatments, based on the average of the two harvest dates.

4. Conclusions

This study demonstrated that the pre-harvest application of AVG in a single dose reduces total phenolics, anthocyanins, and antioxidant activity in the skin of ‘Baigent’ apples grown under anti-hail nets, both at harvest and after cold storage. In contrast, the split AVG application resulted in less pronounced reductions and values closer to the

control, particularly for phenolics and anthocyanins. These findings suggest that split AVG applications may be a preferable strategy for preserving the nutritional and functional quality of apples destined for storage. Future studies should investigate the economic feasibility of multiple applications and assess whether these results are consistent under open-field conditions without anti-hail nets.

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