



## Article

# Relationship between Endogenous Ethylene Production and Firmness during the Ripening and Cold Storage of Raspberry (*Rubus idaeus* 'Heritage') Fruit

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**Abstract:** The raspberry (*Rubus idaeus*) is an important fruit crop; however, its accelerated softening is a critical postharvest problem, even at low temperatures. Its softening has been partially associated with the endogenous production of ethylene from the receptacle during ripening. To understand the relationship between ethylene production and fruit quality at the beginning of the ripening process, the physiological and quality parameters were evaluated during the ripening of the 'Heritage' cultivar. Two storage assays, at 0 °C and 10 °C, were carried out with independent groups of fruits attached to their receptacle at the white stage of fruit development. The treatments included fruit treated with ethylene (1000 ppb) and ethylene perception inhibitor 1-methyl cyclopropene (1-MCP, 1600 ppb) and a control treatment. During ripening, the endogenous production of ethylene in whole fruit was negatively correlated with the loss of firmness. During storage at 0 °C, firmness and ethylene production only decreased by the effect of storage time, with a firmness near 1.5 Newtons at 16 days. On the other hand, the storage at 10 °C showed a delay in the firmness loss and lower ethylene production of the fruit treated with 1-MCP, compared to the control and ethylene-treated fruit. In addition, these two last assays showed a firmness close to 1 Newton at 5 days. No significant differences were observed in the total soluble solids content and titratable acidity between the three treatments at the two storage temperatures. The results during ripening and storage at 10 °C indicate that the loss of the fruit's firmness is positively related to the endogenous ethylene production of the whole fruit from 1 to 5 days of storage. Future assays should be performed to determine the role of ethylene in raspberry ripening.

**Keywords:** receptacle; drupelets; 1-methylcyclopropene; firmness; postharvest

## 1. Introduction

Raspberry (*Rubus idaeus* L.) is a fruit with an extremely short shelf life (1–2 days) due to rapid softening; therefore, it is highly susceptible to damage during harvesting and postharvest operations [1–3]. Although the raspberry has been classified as a non-climacteric fruit, several studies have reported that fruit ripening and abscission are regulated by ethylene

production from the receptacle [1,3–6]. Furthermore, ethylene production is known to increase in raspberry fruit as ripening progresses, leading to a sharp decrease in fruit firmness and shelf life towards maturity [1,3,5–11]. Raspberry fruit development has generally been classified into seven developmental stages, as follows: small green (SG), medium green (MG), large green (LG), white (W), pink (P), red or ripe stage (R), and overripe (OR) fruit [1,3,5,9,11].

The onset of raspberry fruit ripening is at the W stage. In this stage, the red color starts to appear, the titratable acidity decreases, and conversely, the soluble solids content begins to increase [5,7–9]. Additionally, some changes associated with phytohormone metabolism can be observed, such as increases in the production of ethylene from the receptacle, which is associated with the expression of their biosynthetic genes [5,7], and the expression of the genes encoding for the enzyme that conjugate indole acetic acid (IAA) into amino acids [9].

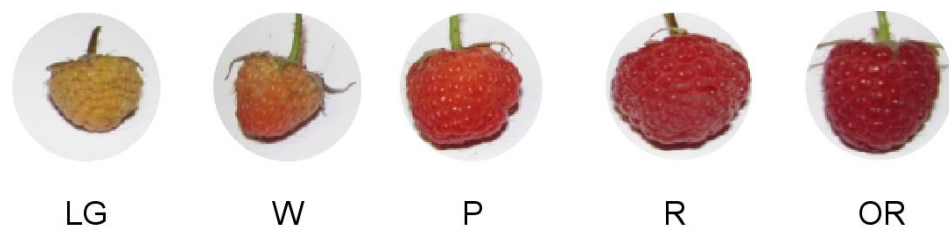
The respiration rate values limit the storage life of the raspberry fruit [12,13]. Therefore, the physiological stage governs the main difference in storage life. It has been reported that the pink stage of different cultivars, such as ‘Heritage’, ‘Santa Catalina’, ‘Santa Clara’ and ‘Santa Teresa’, allows a longer storage life with differences in the loss-of-firmness rate between temperatures [12]. ‘Heritage’ has been described as a cultivar with a dramatic decrease in firmness as the fruit ripens, compared to 28 cultivars [14].

The application of the competitive inhibitor of ethylene, 1-methyl cyclopropene (1-MCP), to green fruit suggests that endogenous ethylene from the receptacle accelerates raspberry abscission and increases both the expression and activity of 1,4-endoglucanase (EGase) in receptacle tissue [3]. Nevertheless, the relationship of endogenous ethylene with the loss of firmness has still been scarcely studied. Thus, with the aim of determining the potential role of ethylene on the changes of fruit quality during ‘Heritage’ raspberry ripening, a cultivar with a high softening rate, the endogenous production of ethylene, and the production of CO<sub>2</sub> and fruit quality parameters (i.e., total soluble solids, titratable acidity, firmness) were evaluated during ripening and under ethylene and 1-MCP application during two storage assays at 0 °C and 10 °C.

## 2. Materials and Methods

### 2.1. Plant Material and Treatments

Raspberry (*Rubus idaeus* L.) ‘Heritage’ fruit was collected from commercial orchards located in Chimbarongo (34°41′45.54 S; 71°10′01.71 W; 333 masl), O’Higgins Region, Chile. The details of the growing conditions have been described previously in Bernales et al., 2019 [9]. Fruit bound to the receptacle and with peduncle were harvested and sorted by size and color [3,5,7–9,11] as follows: large green (LG), white (W), pink (P), red (R) and overripe (OR) fruit (Figure 1). Immediately after harvest, the collected fruits were used to determine the quality and physiological parameters (100 fruits of each stage) under the respective treatments.



### Developmental stages of raspberry Heritage

**Figure 1.** Developmental stages of raspberry (*Rubus idaeus* ‘Heritage’) fruit. The fruit was harvested and sorted by size and color as follows: large green (LG), white (W), pink (P), red (R) and overripe (OR) fruit.

Treatments to determine the role of ethylene were performed using the W-stage fruit (1350 fruits) according to the method of [15]. Briefly, the fruit was put in a clamshell (containing ten whole fruit) and five clamshells were kept in sealed chambers (50 L) for each treatment. The first group was the control group without treatment, the second group was treated by injection with ethylene at 1000 ppb ( $1 \mu\text{L L}^{-1}$  in the chamber), and a third group was treated with 1-methyl cyclopropane (1-MCP; SmartFresh, Rohm and Haas, Vicenza, Italy) by placing tablets of 1-MCP in 20 mL of activator solution to provide a concentration of  $1.6 \mu\text{L L}^{-1}$  in the chamber [15]. First, all fruit was treated in a camera at  $20^\circ\text{C}$  for 16 h. Then, half of the fruit was immediately kept in a growth chamber at  $10^\circ\text{C}$  and the other group was kept in a growth chamber at  $0^\circ\text{C}$  throughout the experiment. The assay was carried out until firmness nearly reached 1 N, and the sample days were 0, 1, 2, 5 days for  $10^\circ\text{C}$  and 0, 1, 2, 5, 9 and 16 days for  $0^\circ\text{C}$  assays. Day 0 was considered as starting after the samples underwent treatments and from 3 h in cold storage. Five independent experimental units (each containing ten whole fruits with an average weight of 2.4 g per fruit) were analyzed for firmness, total soluble solids, titratable acidity,  $\text{CO}_2$ , and ethylene production from each sampling date.

## 2.2. Physiological and Quality Assessments during Ripening

During ripening, the  $\text{CO}_2$  and ethylene production were determined in whole fruits (five experimental units of ten intact fruits binding to the receptacle), drupelets (five experimental units of ten fruits without a receptacle) and receptacles (five experimental units of ten receptacles) of the developmental stages. The drupelets and the receptacle of the LG stage could not be separate, and the  $\text{CO}_2$  and ethylene production were determined only in whole fruit. For this same reason and for the low ethylene production, the SG and MG states were not analyzed in the present study.

The samples of each independent unit for each assay were introduced into close tight chambers (500 mL) and were incubated at  $20^\circ\text{C}$  for 1 h. Furthermore, 1 mL of the gas sample was quantified for ethylene in a gas chromatograph (Shimadzu 8A, Tokyo, Japan) equipped with a flame ionization detector [16]. The results are expressed as microliters of ethylene per kilogram per hour. For  $\text{CO}_2$  determination, the needle of a  $\text{CO}_2$  detector (MAP Headspace gas analyzer, Bridge Analyzers, Bedford Heights, Ohio, USA) was introduced into the same chambers, and the  $\text{CO}_2$  concentrations were recorded. The results are expressed as milligrams of  $\text{CO}_2$  per kilogram per hour.

The firmness of each fruit of the experimental units was measured using FirmTech 2 equipment (BioWorks Inc., Wamego, KS, USA). The firmness was determined as the grams necessary to compress the fruit to 1 mm with a 2.5-cm diameter plunger. Firmness was determined in g/mm and expressed as Newton (N) [16–18]. A total of 8 g of fruit tissue (drupelets) from each experimental unit was homogenized in a mortar. The juice was analyzed for total soluble solids (TSS) using a refractometer (ATAGO, Tokyo, Japan), expressed as the  $^\circ\text{Brix}$  (percentage of sugar per 100 g of the fresh weight (FW) of fruit). The titratable acidity (TA) is expressed as the percentage ( $v/v$ ) of citric acid.

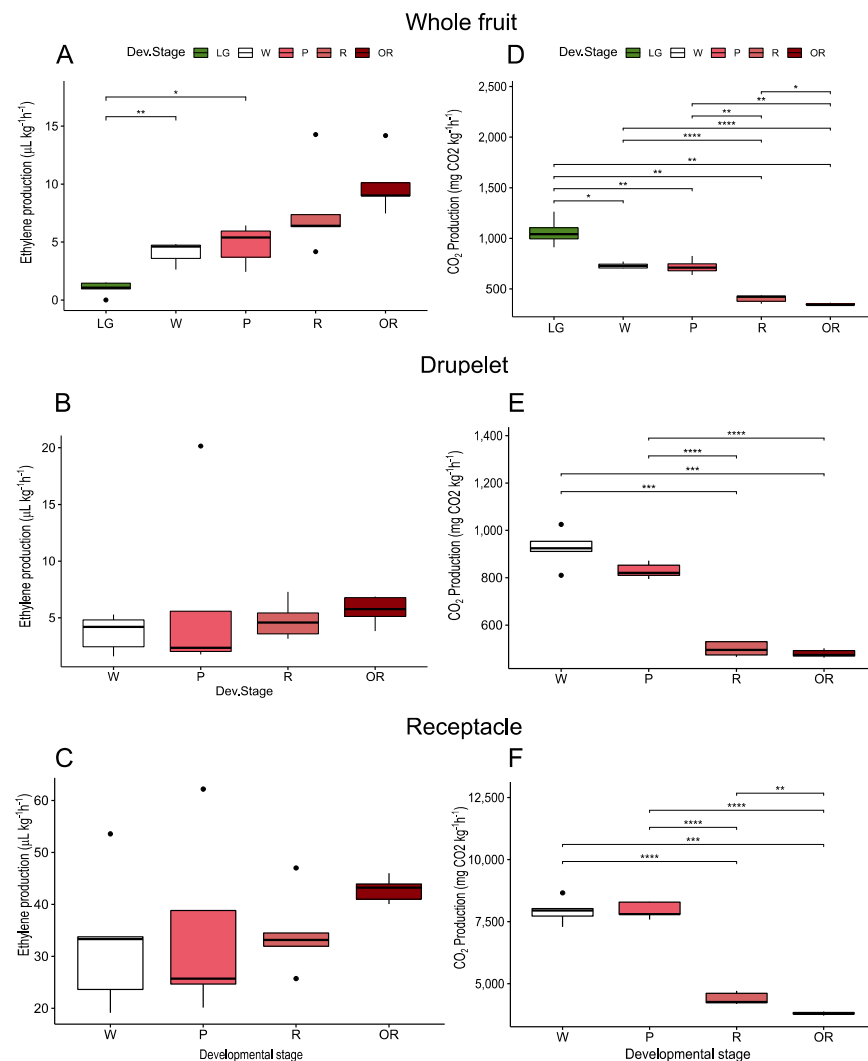
## 2.3. Statistical Analysis

Five independent units (ten fruits) for each sample were used during the development and storage assay (fruit storage in clamshells). The data were analyzed using R statistical software [19]. All results were expressed as the mean  $\pm$  standard deviation (S.D.). The variance in data was analyzed by one-way ANOVA when normality was proved; otherwise, non-parametric techniques were used (Wilcoxon test). Additionally, a principal component analysis (PCA) and correlation matrix (Kendall method) were performed for data. The mean significance differences were determined at  $p \leq 0.05$  (\*),  $p \leq 0.01$  (\*\*),  $p \leq 0.001$  (\*\*\*) and  $p \leq 0.0001$  (\*\*\*\*).

### 3. Results

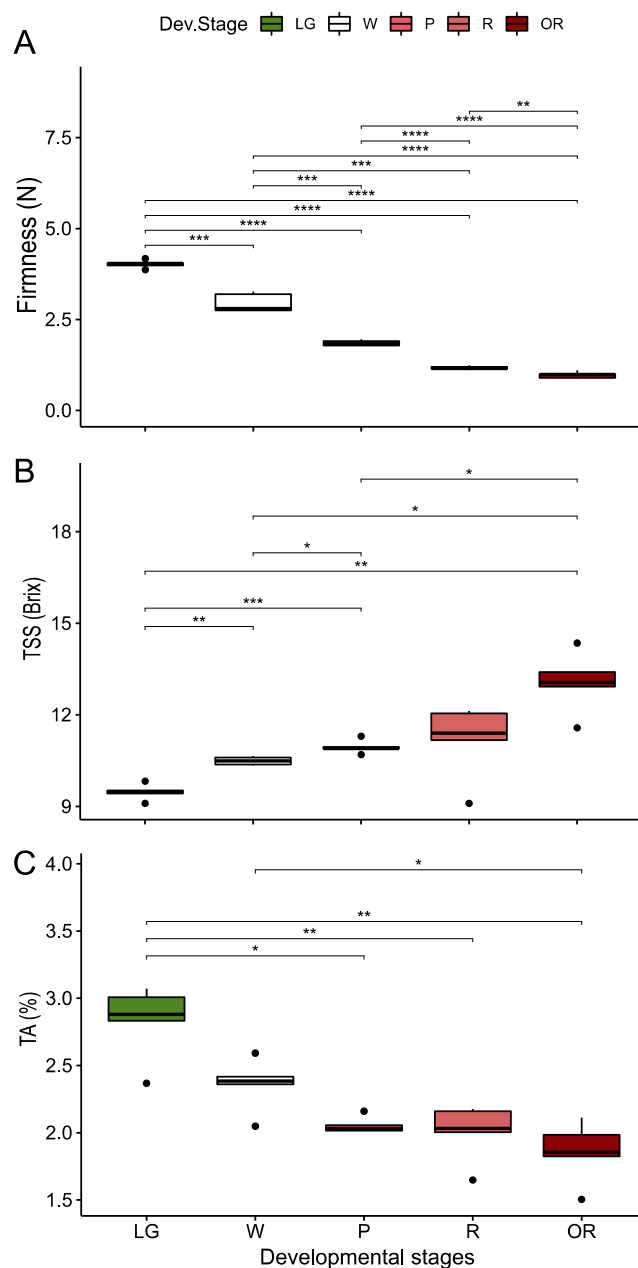
#### 3.1. Physiological and Quality Parameters during the Development of Raspberry Fruit

First, the ripening stages (Figure 1) were characterized by the evolution of ethylene and CO<sub>2</sub> contents. The ethylene production was found to be very low in the whole fruit at the LG stage ( $1.0 \mu\text{L kg}^{-1} \text{h}^{-1}$ ) but increased ten times at the OR stage ( $10.0 \mu\text{L kg}^{-1} \text{h}^{-1}$ ) (Figure 2A). Variable ethylene production was observed in drupelets (Figure 2B). A continuous increase in ethylene production was observed in the receptacle from W ( $32.7 \mu\text{L kg}^{-1} \text{h}^{-1}$ ) to OR ( $42.8 \mu\text{L kg}^{-1} \text{h}^{-1}$ ) stages (Figure 2C), being seven times higher than ethylene produced by drupelets. A constant decrease in CO<sub>2</sub> production was observed for all tissues analyzed, showing the receptacle values to be ten times higher than whole fruit for all stages (Figure 2D–F).



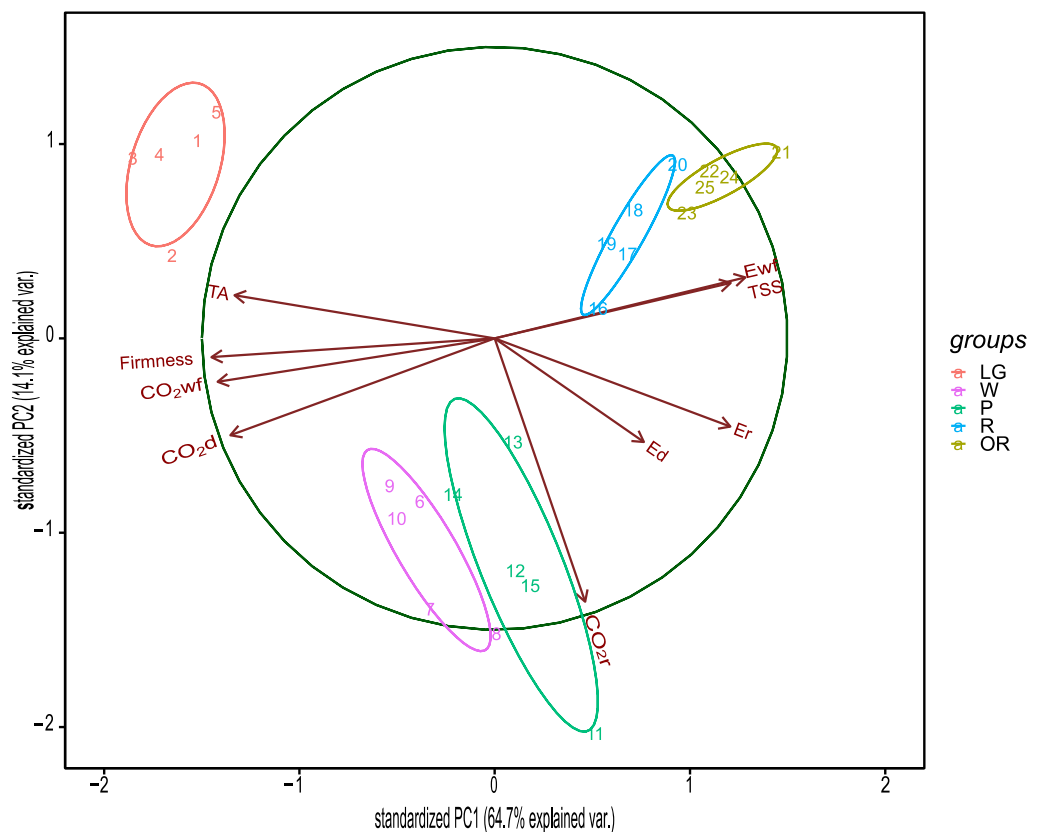
**Figure 2.** Physiological parameters during the development of raspberry (*Rubus idaeus* ‘Heritage’) fruit. The fruit was harvested and sorted by size and color as follows: large green (LG), white (W), pink (P), red (R) and overripe (OR) fruit. Ethylene production ( $\mu\text{L kg}^{-1} \text{h}^{-1}$ ) and CO<sub>2</sub> production ( $\text{mg CO}_2 \text{ kg}^{-1} \text{h}^{-1}$ ) were determined in whole fruit (A,D), drupelets (B,E) and receptacles (C,F) from each stage. The drupelets and the receptacle of the green stage cannot be separated, and the CO<sub>2</sub> and ethylene production were non-determined (n.d) in the drupelets and receptacle. Data represent the means  $\pm$  S.D. from five sample units (each containing ten fruits) by the development stage. Asterisks indicate significant differences between all developmental stages,  $p \leq 0.05$  (\*),  $p \leq 0.01$  (\*\*),  $p \leq 0.001$  (\*\*\*) and  $p \leq 0.0001$  (\*\*\*\*). Boxplots show each group’s distribution (developmental stages), and the black line (central value in the box) signs the median value. Points out of the boxplots show outlier values.

On the other hand, a drastic firmness reduction was observed in the W stage compared to LG (28%) and in the P stage compared to W (34%) (Figure 3A). Moreover, a constant decrease in titratable acidity (TA) and an increase in total soluble solids (TSS) from the LG to OR stages were observed (Figure 3B,C).



**Figure 3.** Quality parameters during raspberry (*Rubus idaeus* ‘Heritage’) fruit development. Firmness (N) (A) was determined in whole fruit, total soluble solids content (TSS, °Brix) (B) and titratable acidity (TA, %) (C) were determined in drupelets. The fruit was harvested and sorted by size and color as follows: large green (LG), white (W), pink (P), red (R) and overripe (OR) fruit. Data represent the means  $\pm$  S.D. from five sample units (each containing ten fruits) by the development stage. Asterisks indicate significant differences between all developmental stages,  $p \leq 0.05$  (\*),  $p \leq 0.01$  (\*\*),  $p \leq 0.001$  (\*\*\*) and  $p \leq 0.0001$  (\*\*\*\*). Boxplots show each group’s distribution (developmental stages), and the black line (central value in the box) signs the median value. Points out of the boxplots show outlier values.

The principal component analysis (PCA) showed that the first two components (PC1 and PC2) explained 64.5% and 14.2%, respectively, of the total variability of the physiological and quality parameters determined during development (Figure 4). In addition, standardized components described a positive correlation between variables, i.e., firmness and CO<sub>2</sub> production in whole fruit (0.81) and the firmness and CO<sub>2</sub> production of drupelets (0.84), and a negative correlation between firmness and ethylene production of whole fruit (−0.73) (Figure 5).



**Figure 4.** The principal component analysis (PCA) biplot for physiological and quality indices of raspberry (*Rubus idaeus* ‘Heritage’) fruit during development. The fruit was harvested and sorted by size and color as follows: large green (LG), white (W), pink (P), red (R) and overripe (OR) fruit. Ewf: ethylene production in whole fruit; Ed: ethylene production in drupelets; Er: ethylene production in the receptacle; CO<sub>2</sub>wf: ethylene production in whole fruit; CO<sub>2</sub>d: ethylene production in drupelets; CO<sub>2</sub>r: ethylene production in the receptacle; TSS: total soluble solids content; and TA: titratable acidity.

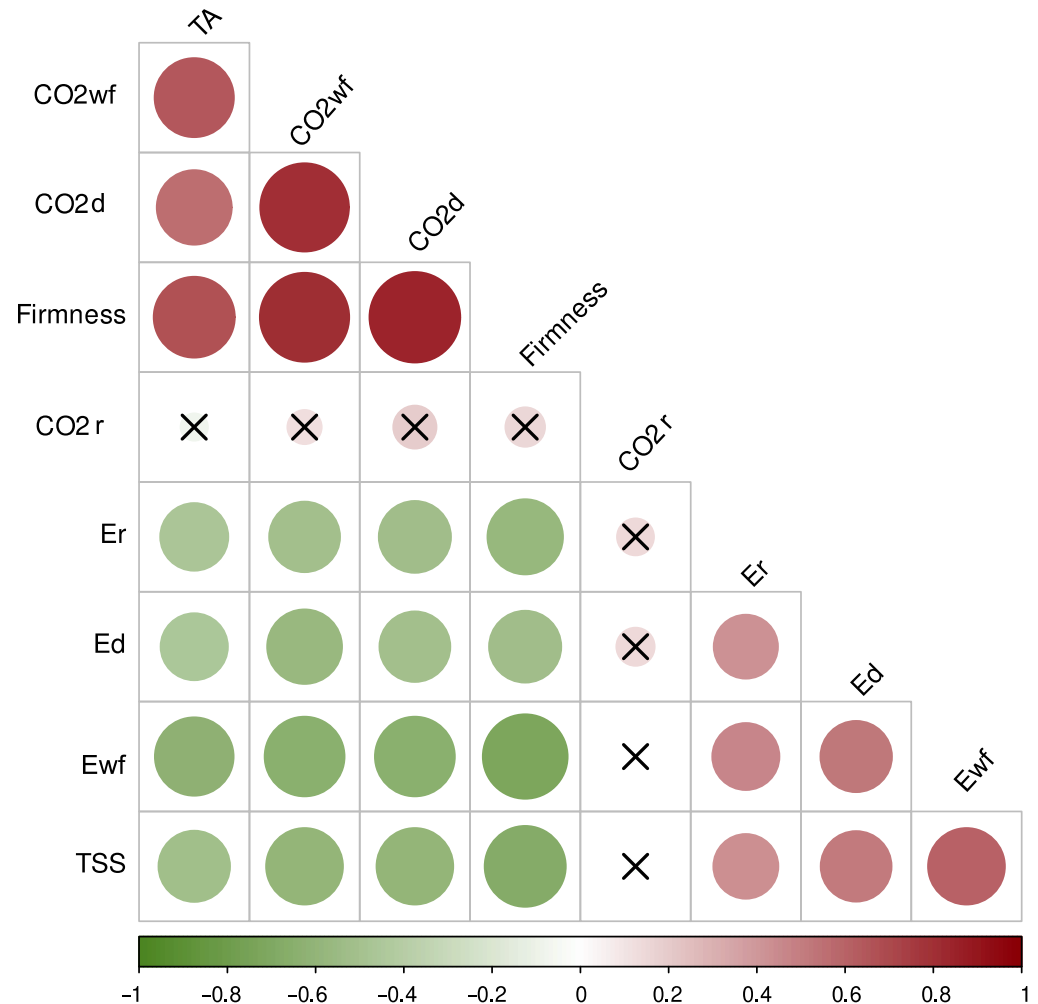
These results indicate that fruit ripening beginning from the W stage gives way to ethylene production in the whole fruit, drupe, and mainly from the receptacle, and quality modifications such as the decrease in fruit firmness and acidity and an increase in TSS. Therefore, the W stage (i.e., transition stage) was chosen to evaluate the effects through the storage assay at different temperatures (0 and 10 °C) of ethylene and 1-MCP application.

### 3.2. Change in Physiological and Quality Parameters during Treatments

#### 3.2.1. Physiological Changes during Treatments

During the assay at 10 °C, significant differences in ethylene production were observed between 1-MCP and the control and ethylene treatments from 1 to 5 d of cold storage (Figure 6A). At 5 days, the fruit reached ethylene production values of 7.66  $\mu\text{L kg}^{-1} \text{h}^{-1}$  for ethylene treatment, 7.76  $\mu\text{L kg}^{-1} \text{h}^{-1}$  for control fruit, and 3.86  $\mu\text{L kg}^{-1} \text{h}^{-1}$  for 1-MCP treatment. Therefore, the 1-MCP treatment decreased the endogenous ethylene production

at half that observed in the control and ethylene-treated fruit at five days of storage at 10 °C. On the other hand, the exogenous ethylene did not increase ethylene production compared to the control fruit.

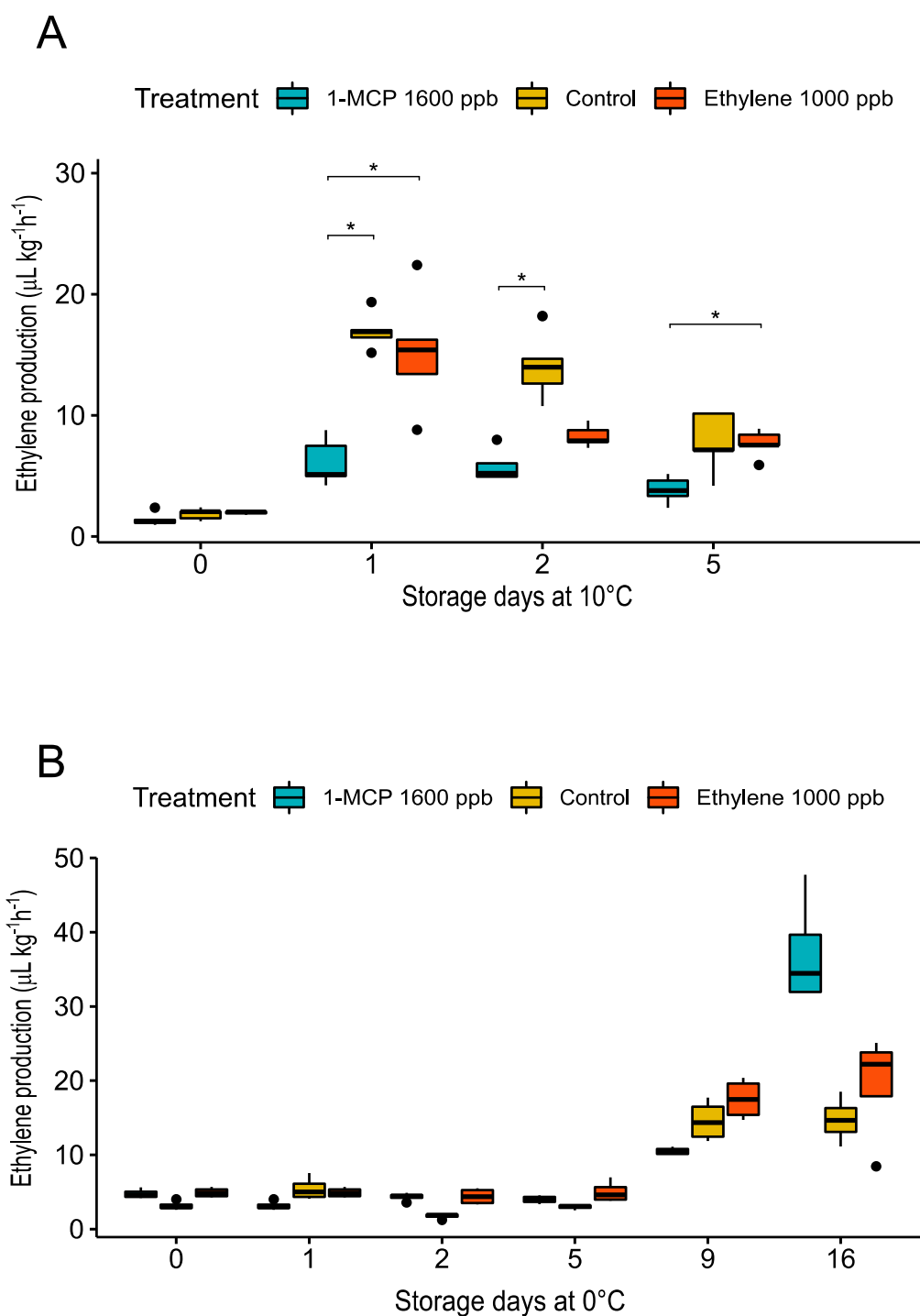


**Figure 5.** Correlation matrix of variables during raspberry (*Rubus idaeus* 'Heritage') fruit development. The correlation was carried out by Kendall's method and on non-grouped data. Ewf: ethylene production in whole fruit; Ed: ethylene production in drupelets; Er: ethylene production in the receptacle; CO<sub>2</sub>wf: ethylene production in whole fruit; CO<sub>2</sub>d: ethylene production in drupelets; CO<sub>2</sub>r: ethylene production in the receptacle; TSS: total soluble solids content; and TA: titratable acidity.

During treatment at 0 °C, ethylene production was kept similar to the harvest ( $4.1 \mu\text{L kg}^{-1} \text{h}^{-1}$ ) up to 5 d, showing significant differences between treatments only at 16 d (Figure 6), observing an ethylene production in the 1-MCP-treated fruit of  $37 \mu\text{L kg}^{-1} \text{h}^{-1}$ , which was 2.5 times higher than the control fruit. At this temperature, endogenous ethylene production was not associated with the inhibition treatment by 1-MCP.

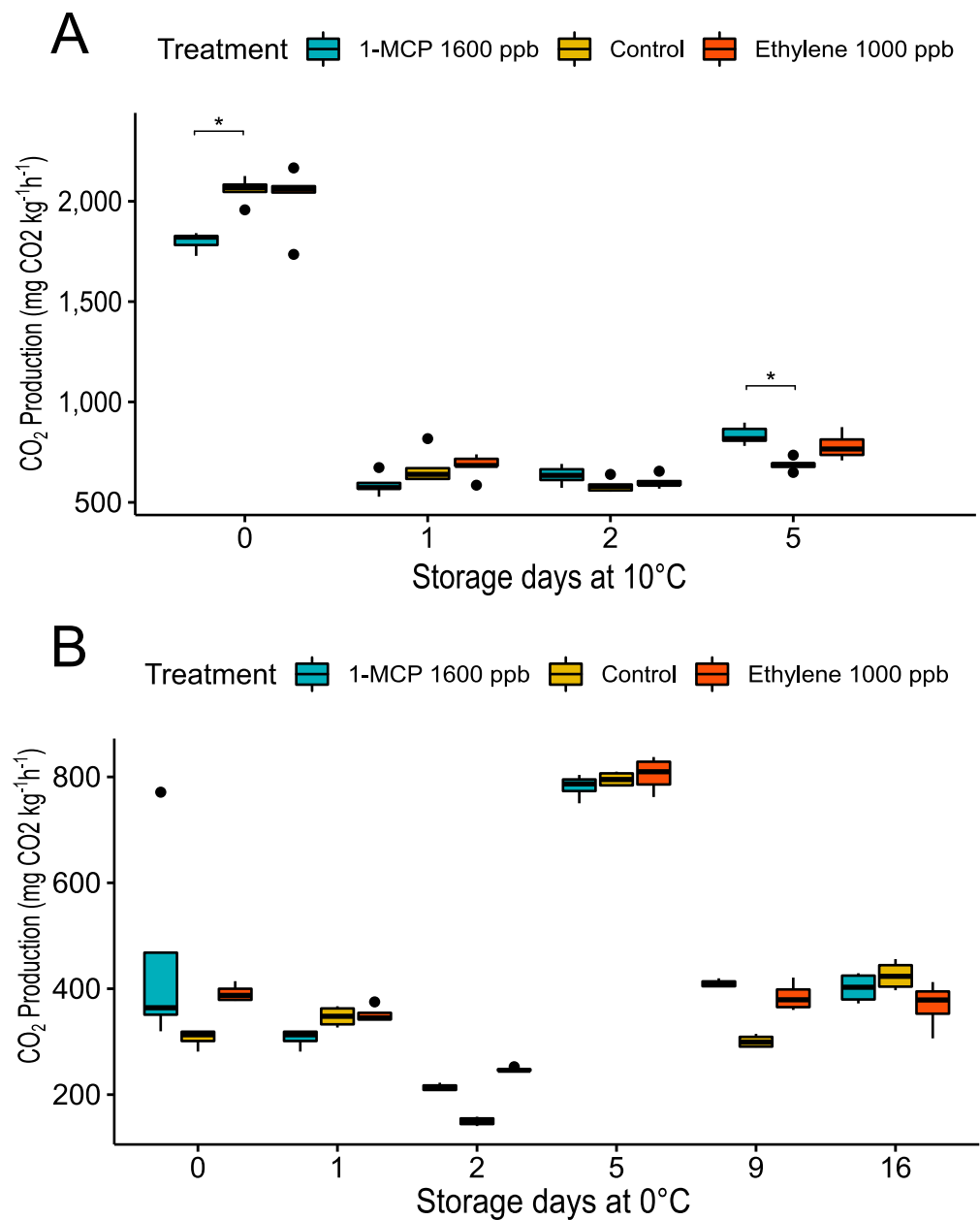
Concerning CO<sub>2</sub> production, the assay at 10 °C showed a similar trend for all treatments, with a significant increase in CO<sub>2</sub> production immediately after treatment application (after harvest at the W stage); with a CO<sub>2</sub> production of 2016, 2056, and 1800  $\text{mg kg}^{-1} \text{h}^{-1}$  for ethylene, control, and 1-MCP treatments, respectively (Figure 7A). In the assay at 0 °C, no significant differences were observed between treatments and a decreasing trend was observed after treatment application up to 2 d. A peak in the CO<sub>2</sub> production was observed at 5 d, and then a decrease was observed at 9 d, maintaining similar values (near  $400 \text{ mg kg}^{-1} \text{h}^{-1}$ ) until 16 d (Figure 7B).





**Figure 6.** Ethylene production of raspberry (*Rubus idaeus* ‘Heritage’) fruit during treatment with ethylene and 1-methyl cyclopropane (1-MCP). The storage assay was performed at the W stage of raspberry and stored for 5 and 16 days at 10 °C (A) and 0 °C (B), respectively. Ethylene production ( $\mu\text{L kg}^{-1}\text{h}^{-1}$ ) was determined for ethylene and 1-MCP-treated fruits and controls. Data represent the means  $\pm$  S.D. from five sample units (each containing ten fruits). Asterisks indicate significant differences between treatments at the same storage time ( $p \leq 0.05$ ). Boxplots show each group’s distribution (treatment) and the black line (central value in the box) signs the median value. Points out of the boxplots show outlier values.



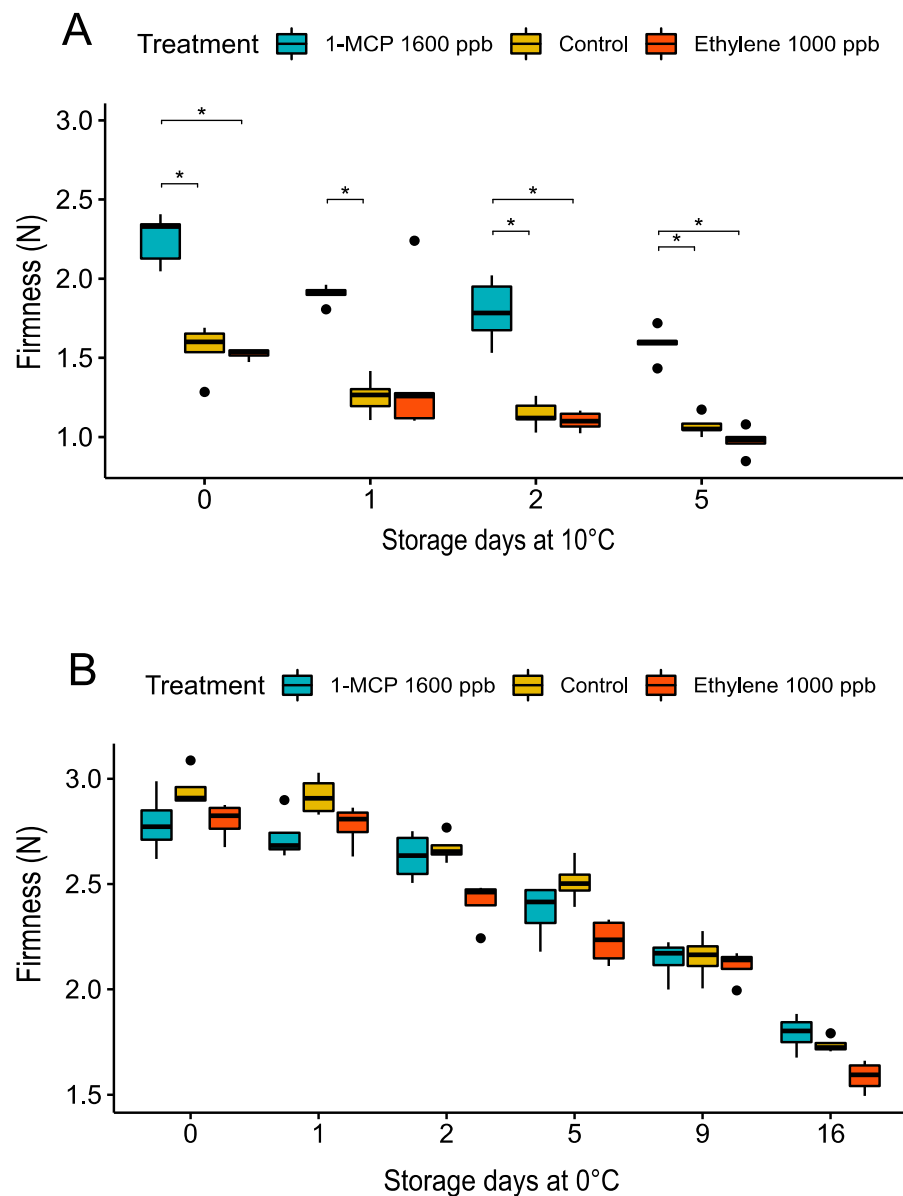


**Figure 7.** CO<sub>2</sub> production of raspberry (*Rubus idaeus* ‘Heritage’) fruit during treatment with ethylene and 1-methyl cyclopropane (1-MCP). The storage assay was performed at the W stage of raspberry and stored for 5 and 16 days at 10 °C (A) and 0 °C (B), respectively. CO<sub>2</sub> production (mg CO<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup>) was determined for ethylene and 1-MCP-treated fruits and controls. Data represent the means  $\pm$  S.D. from five sample units (each containing ten fruits). Asterisks indicate significant differences between treatments at the same storage time ( $p \leq 0.05$ ). Boxplots show each group’s distribution (treatment) and the black line (central value in the box) signs the median value. Points out of the boxplots show outlier values.

### 3.2.2. Firmness Changes during Treatments

During the assay at 10 °C, significant firmness differences were observed between 1-MCP compared to control and ethylene treatments during each time evaluated at this cold storage temperature, except for day 1, where there were no significant differences between 1-MCP and ethylene-treated fruit due to an outlier point. At 5 days under this storage temperature, the fruit reached firmness values of 0.97, 1.07, and 1.59 N for ethylene, control, and 1-MCP treatments, respectively. Therefore, the ethylene receptor inhibition allowed

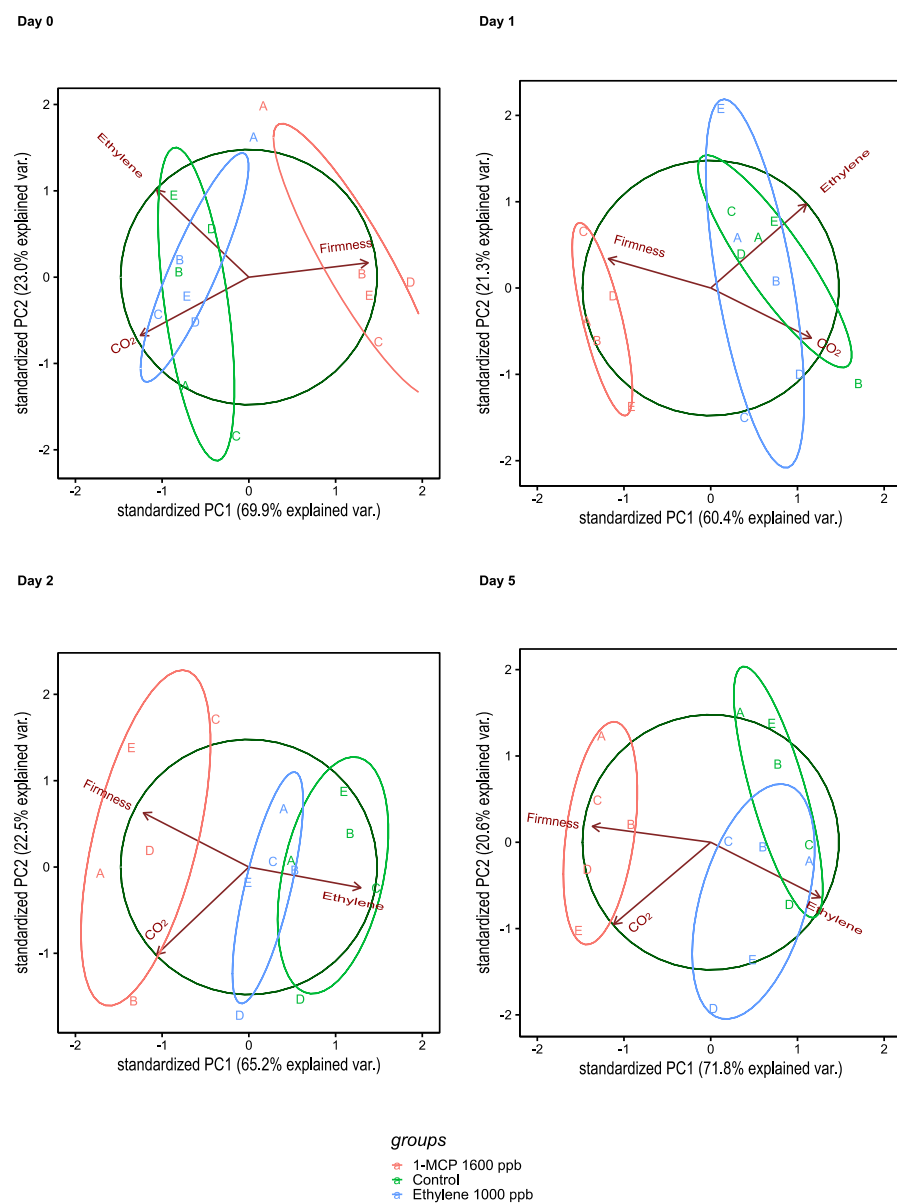
for maintaining 55% of the firmness determined at the harvest, suggesting a relation of endogenous ethylene production with the firmness (Figure 8A). Nevertheless, 1-MCP treatment did not preserve the firmness above 2 N even on the first day of storage and failed to prolong the storage of the fruit for more than 5 days, with temperature being a key factor for the preservation of fruit firmness.



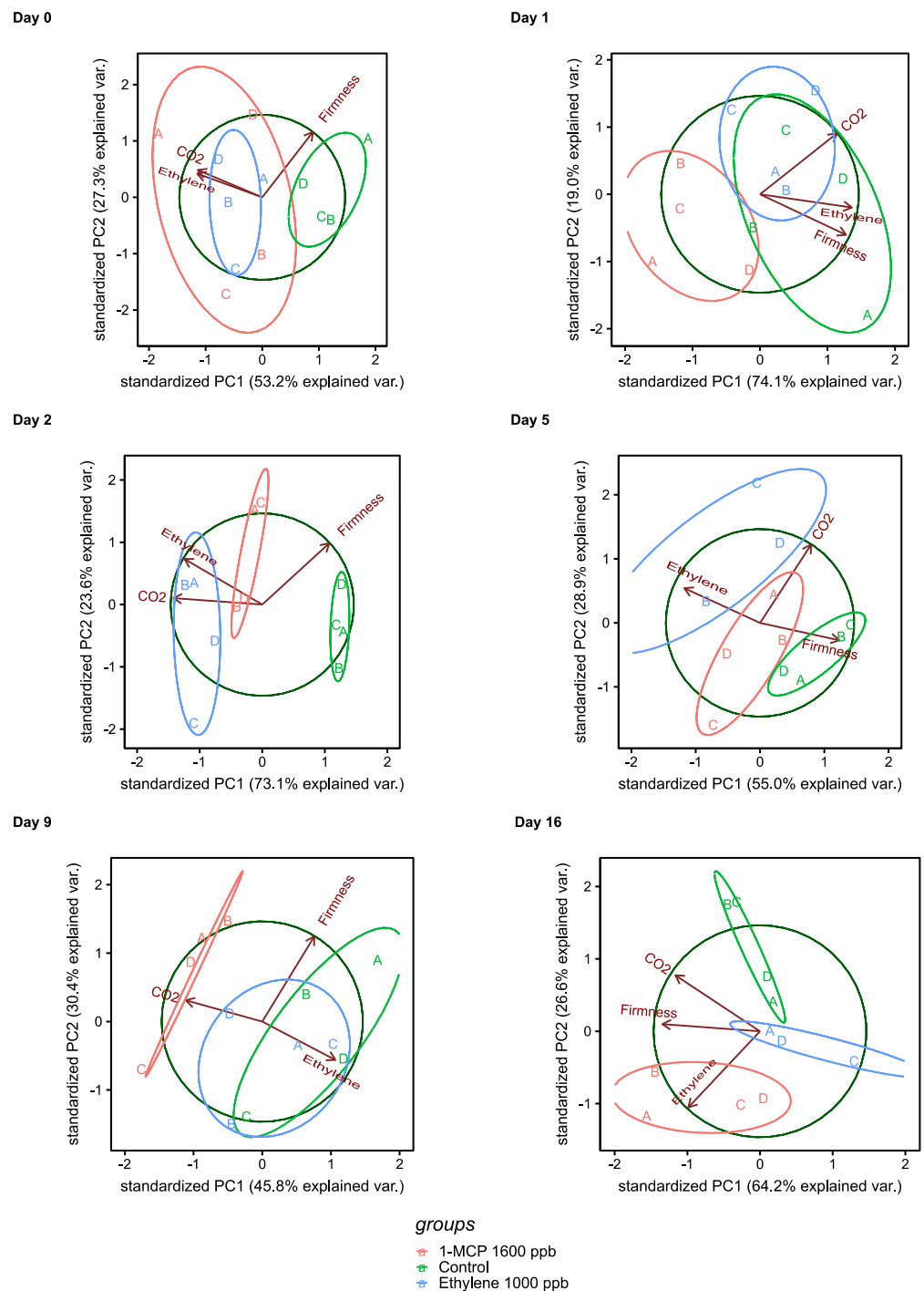
**Figure 8.** The firmness of raspberry (*Rubus idaeus* ‘Heritage’) fruit during treatment with ethylene and 1-methyl cyclopropane (1-MCP). The storage assay was performed at the W stage of raspberry fruit and stored for 5 and 16 days at 10 °C (A) and 0 °C (B), respectively. Firmness (N) was determined for ethylene and 1-MCP-treated fruits and controls. Data represent the means  $\pm$  S.D. from five sample units (each containing ten fruits). Asterisks indicate significant differences between treatments at the same storage time ( $p \leq 0.05$ ). Boxplots show each group’s distribution (treatment) and the black line (central value in the box) signs the median value. Points out of the boxplots show outlier values.

During treatment at 0 °C, the fruit showed a firmness over 2 N for up to 9 days. No significant differences were observed between 1-MCP treatment, control and exogenous ethylene-treated fruit at this cold storage temperature (Figure 8B). At this temperature, no effect on fruit firmness was observed by inhibiting the ethylene receptor.

The principal component analysis (PCA) was conducted for each day to better visualize variable behavior by day. The PCA showed that the first two components (PC1 and PC2) explained more than 60% and more than 20%, respectively, of the variability, i.e., ethylene production, CO<sub>2</sub> production and firmness determined between treatments at 10 °C storage temperature (Figure 9). At this temperature, the 1-MCP treatment behaved independently of the control and ethylene-treated fruit groups during all sampling days' analyses. At 10 °C, standardized components described a low negative correlation between firmness and CO<sub>2</sub> production at 0 days (0.43) and a low positive correlation at 5 days (0.24). At this temperature, there was a low negative correlation between firmness and ethylene production at 0 days (−0.24), increasing partially at 5 days (−0.54) (Figure 10). These results suggest that although the 1-MCP group had an independent behavior from the other treatments, the firmness relationship with the production of whole fruit ethylene during this assay was partial.



**Figure 9.** PCA biplot for ethylene, CO<sub>2</sub> and firmness indices of raspberry (*Rubus idaeus* 'Heritage') fruit during treatment with ethylene and 1-methyl cyclopropane (1-MCP) at 10 °C. The storage assay was performed at the W stage of the raspberries and stored for 5 days and at 10 °C. The PCA analysis was conducted on each sampling day.

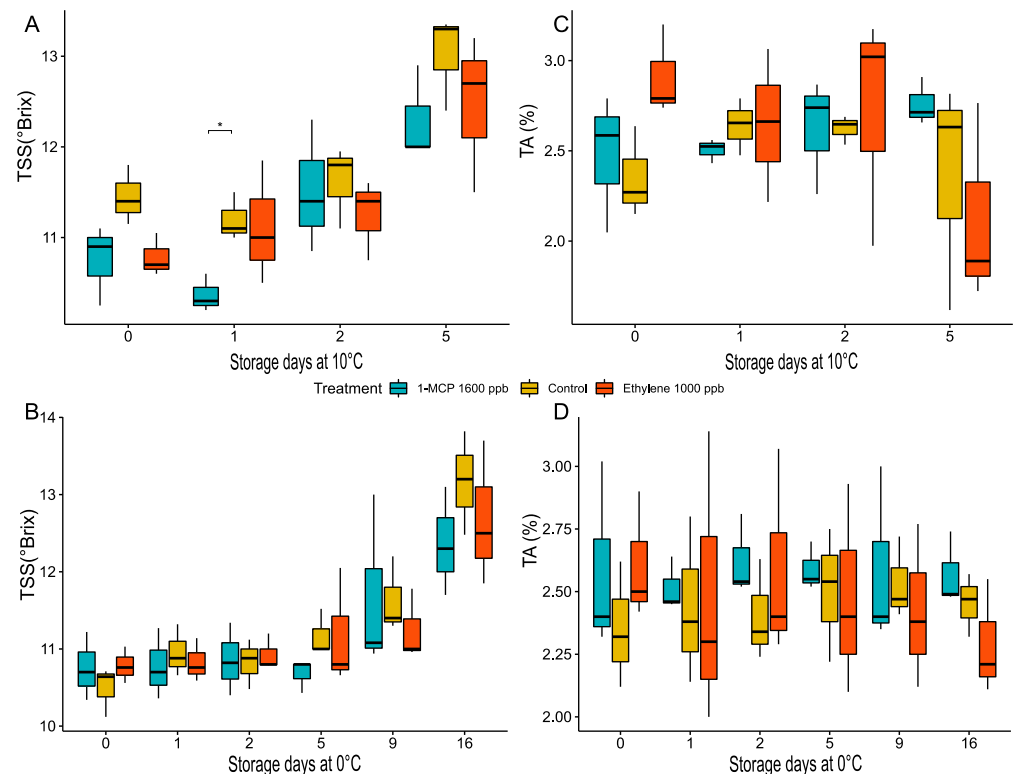


**Figure 10.** PCA biplot for ethylene, CO<sub>2</sub> and firmness indices of raspberry (*Rubus idaeus* 'Heritage') fruit during treatment with ethylene and 1-methyl cyclopropane (1-MCP) at 0 °C. The storage assay was performed at the W stage of the raspberry fruit and stored for 16 days and at 0 °C. The PCA analysis was carried out on each sampling day.

The principal component analysis showed that the first two components (PC1 and PC2) explained more than 45% and more than 19%, respectively, of the variability in ethylene production, CO<sub>2</sub> production and firmness, determined between treatments at a storage temperature of 0 °C (Figure 10). At this temperature, the 1-MCP-treated fruit was independent of the control and the ethylene-treated fruit was only at 9 and 6 days of storage (Figure 10), confirming that the treatment with 1-MCP was ineffective at 0 °C.

### 3.2.3. Total Soluble Solids (TSS) and Titratable Acidity (TA) Changes during Treatments

During the cold storage assay, no significant differences in TSS were observed between treatments independent of the temperature of the assay (Figure 11). During the assay at 10 °C (Figure 11A), the fruit reached values of 12.6 °Brix at 5 days of storage, whereas the fruit under 0 °C reached similar TSS values after 16 days of storage, independent of the treatment (Figure 11B). Therefore, the TSS increased 1.2 times to harvest value after 5 days of storage at 10 °C and after 16 days at 0 °C. The TA values (average 2.5%) during the different treatments and cold storage temperature (Figure 11) remained similar to those obtained in the harvest (2.4%).



**Figure 11.** The total soluble solids and titratable acidity of raspberry (*Rubus idaeus* ‘Heritage’) fruit during treatment with ethylene and 1-methyl cyclopropane (1-MCP). The storage assay was performed at the W stage of the raspberry fruit and stored for 5 and 16 days at 10 °C (A,C) and 0 °C (B,D), respectively. Total soluble solids (TSS, °Brix) (A,B) and titratable acidity (TA, %) (C,D) were determined for ethylene and 1-MCP-treated fruits and controls. Data represent the means  $\pm$  S.D. from five sample units (each containing ten fruits). Asterisks indicate significant differences between treatments at the same storage time ( $p \leq 0.05$ ). Boxplots show each group’s distribution (treatment), and the black line (central value in the box) signs the median value. Points out of the boxplots show outlier values.

## 4. Discussion

In this study, the physiological and quality parameters of ‘Heritage’ raspberry fruit were evaluated during its development, showing a continuous increase in ethylene production in the receptacle, at least sevenfold higher than that produced by drupelets at R and OR stages. Conversely, a constant decrease in CO<sub>2</sub> production was observed for all tissues analyzed from LG to OR stages, ten times higher in the receptacle than whole fruit for all stages. This ethylene production pattern with different respiratory rates can be observed in raspberries (‘Heritage’) grown in the same location during three seasons [5,9]. Furthermore, a negative correlation between ethylene and fruit firmness has been described during the ripening of the whole fruit of this cultivar [7], as observed in the present analysis of PCA and the correlation matrix (Figures 4 and 5) for the whole fruit. In the present and previous

studies [5,9], the continuous ethylene production observed in raspberry fruit is not similar to other non-climacteric species. For example, in strawberry, the ethylene production was higher only in the green fruit stage, decreasing in white (W) stage fruit, and then showed an increasing trend towards the red stage, concomitant with an enhanced respiration rate [20].

Previous studies have indicated the W stage of fruit development as the onset of 'Heritage' raspberry ripening [5–9]. Moreover, the W stage has been described as the first stage of ripening in the three new Chilean raspberry cultivars: 'Santa Catalina', 'Santa Clara' and 'Santa Teresa' [12]. It has been described that 'Heritage' showed a sharp decline in fruit firmness throughout the developmental stages compared to 'Santa Catalina', 'Santa Clara' and 'Santa Teresa' cultivars [12]. In these four cultivars, the ethylene production rate measured one day after harvest was always the highest at the dark red stage and lowest in the less mature fruit at the pink stage [12]. Therefore, the drastic decline in firmness and increase in ethylene production observed at the W stage (Figures 2 and 3) led us to analyze the relationship between this fruit quality parameter and the hormone.

In the present study, the 1-MCP reduced the ethylene production compared to control and ethylene treatments only at 10 °C. At 0 °C, ethylene production was kept similar to the harvest for a duration of five days, independent of treatment (Figure 6), suggesting that 'Heritage' maintains a metabolic rate at 10 °C, where the response to ethylene is clearly perceived compared to the fruit stored at 0 °C. Furthermore, our results obtained for 1-MCP-treated 'Heritage' fruit stored at 10 °C are consistent with the decreased ethylene production reported in strawberry following 1  $\mu\text{L L}^{-1}$  1-MCP exposure and stored at 5 °C [21].

The increase in ethylene and decrease in CO<sub>2</sub> production during the ripening and the reduction in ethylene production by 1-MCP application, with a non-effect in CO<sub>2</sub> production at 10 °C, indicate both a climacteric behavior of ethylene production, similar to tomato [22], and a non-climacteric production of CO<sub>2</sub>. The decreasing trend of CO<sub>2</sub> of raspberry fruit coincides with the non-climacteric fruit behavior and previous studies of different raspberry cultivars, including 'Heritage' [8,12]. On the other hand, the constant increase in ethylene in whole fruit during ripening is very different from that in other non-climacteric fruit, such as grape [23,24] and strawberry fruits [20], with ethylene peaks during early development, a typical pattern in non-climacteric fruit. However, some outlier points in the determination of ethylene production during development (Figure 2) make it necessary to deepen the understanding of ethylene production during raspberry ('Heritage') fruit development, for example, using chambers for ethylene determination of fruit in planta. It has been reported that an anti-sense suppression of the gene encoding for *Malus domestica*, 1-amino-cyclopropane-carboxylase oxidase (MdACO1), resulted in fruit with early ripening events (conversion of starch to sugars), showing a low dependency for ethylene, but a high sensitivity to low concentrations of ethylene (0.01  $\mu\text{L L}^{-1}$ ). By contrast, late ripening events (flesh softening) showed a high dependency for ethylene but were less sensitive to low concentrations (needing 0.1  $\mu\text{L L}^{-1}$  for a response) [25].

The extremely short shelf-life of raspberry fruit (1–2 days) is one of the most critical limitations during harvest and postharvest operations [1,3,26]. In strawberry fruit (*F. × ananassa* 'Oso Grande'), it has been reported that 1-MCP delayed the softening of the fruit, since fruit treated with 100 nL L<sup>−1</sup> 1-MCP showed greater firmness and a minor percentage of pectin solubilization during storage at room temperature [27]. In *F. × ananassa* 'Toyonoka', the application of ethylene (2 mmol L<sup>−1</sup> ethephon) did not modify the activity of cell wall hydrolases related to softening (e.g., polygalacturonase), whereas treatment with 1  $\mu\text{L L}^{-1}$  1-MCP clearly did [28]. In the present study, the ethylene receptor inhibition by 1-MCP treatment during storage at 10 °C allowed for maintaining 55% of the firmness determined at the harvest, suggesting a relation of endogenous ethylene production with the firmness at this temperature (Figure 8A). Nevertheless, this treatment does not preserve the firmness observed at harvest and fails to prolong the storage of the fruit for more than 5 days. Under 0 °C, the fruit showed a firmness (over to 2 N) for up to 9 days. No significant differences were observed for 1-MCP treatment compared to the control and

ethylene-treated fruit at this cold storage temperature (Figure 8B). At this temperature, it is likely that the low metabolic rate allows a lower response to ethylene and, therefore, a lower effect of the ethylene receptor inhibitor. In ‘Selva’ strawberry, Ku et al. [29] describe that the 1-MCP treatment at a lower concentration (5 to 15 nL·L<sup>-1</sup>) extended its postharvest life (i.e., freshness or gloss, firmness and mold absence) by 35% and 150% at 20 °C and at 5 °C, respectively. On the other hand, the same authors [29] indicated that higher 1-MCP concentrations (500 nL·L<sup>-1</sup>) accelerated the loss of quality (60%) of four strawberry cultivars at 5 °C. Therefore, the partial effect of 1-MCP on the maintenance of raspberry fruit’s firmness may be due to the 1-MCP concentration.

Other key phytohormones probably regulate raspberry firmness that is associated with ethylene. In non-climacteric fruit, abscisic acid (ABA) has been described as the main hormone that regulates the ripening process [6]. Early in the 1980s, ABA was reported to be involved in strawberry ripening [30] and has been defined as the main regulator of the onset of grape berry ripening [31], regulating the softening, coloration and sugar accumulation [31–34]. On the other hand, it has been suggested that ethylene plays a secondary role in strawberry ripening compared to abscisic acid. Therefore, ethylene can regulate specific events related to the ripening process in strawberries and grapes, such as coloration. In grape (*Vitis vinifera* ‘Cabernet Sauvignon’), it has been described that ethylene increases anthocyanin content [35]. The ethylene effect depends on the developmental stage and species evaluated in strawberries. It has been reported that unripe fruit of *Fragaria chiloensis* (large green stage) treated with ethephon showed a lower anthocyanin content but a higher lignin content compared to the control at 48 and 216 h after treatment [36]. These findings contrast with the results observed in *F. × ananassa* fruit (‘Toyonoka’ and ‘Camarosa’), where ethylene treatment at the white developmental stage promoted the accumulation of anthocyanins [28,37], and the application of 1-MCP [28] decreased that process. To date, no changes in ABA levels have been described during ripening, but it is certainly a possible candidate to regulate raspberry ripening.

The bright red stage of the ‘Heritage’ raspberry has been described with a low sugar/acid ratio (3.8) [12,38]. However, in the present study, fruit at the red stage shows an average total soluble solids (TSS) content (11.2 °Brix) (Figure 3B) higher than previously described by Contreras et al., 2021 [12] for ‘Heritage’ (8.2 °Brix) harvested in winter in Santo Domingo (33°38′09″ S, 71°37′41″ W), Valparaíso Region, Chile. Our result related to the TSS value was similar to that described for ‘Santa Catalina’ raspberry fruit (10.6 °Brix) [12]. These differences may be due to the month of harvest and localities, where the summer–autumn season favors the accumulation of soluble solids content. A constant decrease in titratable acidity (TA), and an increase in TSS content were observed during development (Figure 3B,C). In fact, TSS (0.60) and TA (−0.63) did not show a high correlation, positive or negative, with the ethylene production from whole fruit during development (Figure 5). On the other hand, a TSS content increase was observed only in the final days of each assay, without marked differences between treatments at both temperatures (Figure 11). Conversely, TA remained similar to those obtained in the harvest during all storage times, and no significant differences were observed between treatments, independent of storage temperature (Figure 11). These findings are similar to those described by Perkins-Veazie and Nonnecke (1992) [8], with a TSS content and TA independent of ethylene levels in ‘Heritage’ raspberry. In the present study, no correlation was observed between TSS and TA with ethylene production from whole fruit during development (Figure 5). Therefore, TSS and TA changes can be regulated by an independent ethylene mechanism. Similarly, in *F. × ananassa* ‘Pajaro’, ethylene (100 µL L<sup>-1</sup>) treatment showed no changes in soluble solids content [39].

In ‘Pajaro’ strawberry fruit, ethylene treatment showed that fruit softening and color development were slightly accelerated by ethylene (100 µL L<sup>-1</sup>) treatment, stimulating the respiration in early-harvested strawberry fruit within 2 days. The 1-MCP affected the respiratory rise induced by exogenous ethylene dependent on fruit maturity [39]. In the present study, the fruit treated with 1-MCP and stored at 10 °C presented a lower loss of



firmness associated with lower ethylene production during the days of storage, compared to the control fruit and ethylene-treated fruit. The results indicate that the firmness of the fruit stored at 10 °C partially depends on the endogenous production of ethylene, but does not respond to exogenous ethylene application. The PCA showed that the 1-MCP treatment behaved as an independent group to the control and ethylene-treated fruit at that temperature, but with a low negative correlation between firmness and ethylene production. These results suggest a partial effect of the endogenous ethylene from the receptacle on the firmness loss of the whole fruit.

## 5. Conclusions

In the present study, a decrease in firmness and titratable acidity (TA) with increased total soluble solids (TSS) was observed through ripening. The increase in ethylene production with a negative correlation with a decrease in the firmness of whole fruit (−0.73) during ripening and the effect of 1-MCP application suggests an effect of endogenous ethylene mainly over firmness. Moreover, the positive correlation between firmness and CO<sub>2</sub> production in the whole fruit (0.81) suggests a ripening behavior for ‘Heritage’ raspberry fruit that could be placed between climacteric and non-climacteric behavior. On the other hand, the lack of correlation, positive or negative, between TSS and TA with ethylene production from whole fruit during development, and the lack of effect of 1-MCP treatment on TSS and TA, suggest an ethylene-independent regulation mechanism of sugars and organic acid accumulation. Therefore, even though endogenous ethylene could partially regulate the firmness of ‘Heritage’ raspberry fruit detached from plants, more information must be gathered from field assays to determine the regulatory role of ethylene on the loss of firmness during ripening, along with its crosstalk with other hormones and the impact of these applications during storage.

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