

# Late summer pruning improves the quality and increases the content of functional compounds in Fuji apples

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**ABSTRACT:** The objective of this paper was to evaluate the effect of the time of summer pruning on the quality of Fuji apples, on the red color and functional properties, at harvest and after 3.5 months of cold storage. The treatments evaluated consisted of the control (without summer pruning) and summer pruning in December, January, and February. The percentage of fruit with more than 50% red color on the skin was 20.5% higher when summer pruning was performed later than when performed early. In general, summer pruning in January and February provided fruit with a relatively high content of anthocyanins (19 and 25.1 mg cyaniding 3-glycoside·100 g<sup>-1</sup> FW, respectively), relatively high values of total phenolic compounds (370.1 and 438.7 mg EAG·100 g<sup>-1</sup> FW, respectively) and total antioxidant activity by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method (8,097.5 and 8,089.2 μMol Trolox·100 g<sup>-1</sup> FW, respectively) in the 2017/18 season. Summer pruning did not affect fruit quality after cold storage. Summer pruning performed in January or February increased functional compounds content and improved red color, while in February it increased flesh firmness.

**Key words:** *Malus domestica* Borkh., anthocyanins, phenolic compounds, antioxidant activity, human health.

## INTRODUCTION

The physicochemical attributes of apples are important aspects in defining their monetary value and consumer acceptance of the product. Sugars, organic acids, and their relationship (ratio) are essential to fruit flavor (Petkovsek et al. 2007). However, the production of fruit without defects and with a high value and expressive red color is decisive for their classification in high-value categories, which impacts the profits of the producer and meets the preference of the consumer market (Fiovaranço and Lazzarotto 2012).

The regular consumption of apples improves human health, mainly by protecting against chronic diseases (Condezo-Hoyos et al. 2014). Polyphenols, found in apples, play an important role in the human health-promoting and anthocyanins, reduce the oxidation of low-density lipoprotein (LDL) cholesterol and decrease the development and progression of atherosclerotic lesions (Cardoso et al. 2011, Cory et al. 2018).

A few environmental factors, such as light, temperature, and humidity, and agronomic practices, such as irrigation, nutrition, thinning, and pruning, affect and change the quality attributes of apples during preharvest (Musacchi and Serra 2018). Highly dense orchards, with tall and vigorous plants, tend to have decreased light penetration in the canopy, especially in the lower parts and between plants, resulting in relatively low fruit yield and quality (Djordjević et al. 2020). Environments with high sunlight irradiation are favorable to the production of relatively large fruit with a high percentage of dry matter and red coloring (Musacchi and Serra 2018). Improving fruit quality by changing the canopy environment,

especially regarding the amount of incident light, is among the benefits of summer pruning of apple trees (Cooley and Autio 2011). The greater availability of light in plants subjected to summer pruning allows for enhanced red coloring, anticipation of maturation, reduction in titratable acidity, and increased soluble solid contents of the fruit (Ashraf and Ashraf 2014).

Information relating to the time in which pruning is performed and its effects on the physicochemical and functional quality of apples is scarce. The time of summer pruning, in addition to influencing the physical-chemical characteristics, possibly has an influence on the functional quality of the fruits. Thus, the objective of this paper was to evaluate the effect of the time of summer pruning on the quality of Fuji apples, especially on the red color and functional properties.

## MATERIAL AND METHODS

The experiment was carried out in an experimental orchard located at the São Joaquim Experimental Station, belonging to the Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina (EPAGRI) (28°17'39" S, 49°55'56" W; altitude of 1,415 m), São Joaquim, SC, Brazil, during the crop seasons of 2016/2017, 2017/2018, and 2018/2019. Apple trees of the Fuji Standard cultivar on Marubakaido rootstock with M9 filter were utilized. The orchard was established in 1999, with 4 m between rows and 1.5 m between plants.

The region's climate is humid mesothermal (Cfb), according to the Köppen-Geiger classification. In other words, the region has a constantly humid, temperate climate, with no dry season and cool summer. The average accumulation of temperatures of 7.2°C or less in the region is 900 hours. The soil of the experimental field was classified as Humic Cambisol, according to the Brazilian Soil Classification System (Alvares et al. 2014, Pasa et al. 2018). Figure 1 shows the data on climatic conditions, average temperature, thermal amplitude, rainfall, and hours of insolation from December to April in the three crop seasons evaluated.

Treatments consisted of different times of summer pruning (December, January, and February), performed on the 15<sup>th</sup> of each month, by removing whole branches in the upper, middle, and lower positions of the vegetative canopy, and without summer pruning (control). Vertical branches, and those that shaded the fruit, were removed by summer pruning. The experimental design used was a randomized blocks design with four replicates and an experimental unit composed of three plants.

Fruit of all treatments were harvested on April 26, 2017, April 11, 2018, and April 15, 2019, and transported to the laboratory for analysis of maturation and quality attributes. The following variables were evaluated: percentage of fruit with more than 50% red color, skin color (red color and base color), iodine-starch index, flesh firmness, soluble solids (SS), titratable acidity (TA), and the SS/TA ratio. In the 2016/17 and 2017/18 crop seasons, apple skins were sampled to analyze the contents of anthocyanins, total phenolic compounds (TPC), and total antioxidant activity (TAA; ABTS and DPPH methods). Fifty fruit per experimental unit were used to evaluate the percentage of fruit with more than 50% red color, while 20 fruit were used for the other evaluations. Thirty fruit per experimental unit of the 2016/12 and 2017/18 crop seasons were stored for 3.5 months ( $1 \pm 0.2^\circ\text{C}$  and relative humidity of  $92 \pm 4\%$ ) plus seven days of shelf life ( $23 \pm 3^\circ\text{C}$  and relative humidity of  $60 \pm 5\%$ ), simulating the marketing period. The apples were subsequently evaluated for flesh firmness, skin color (base color, changing from green to yellow), SS, TA, SS/TA ratio, and rot incidence.

### Physicochemical attributes evaluation

- The percentage of fruit surface covered with red coloring was subjectively and visually assessed, counting number of fruit with a surface covered with more than 50% red color;
- Skin color was measured in terms of hue angle ( $h^\circ$ ) in the more and less red regions of the fruit (skin background color), using a colorimeter (Konica Minolta® CR 400, Tokyo, Japan);
- Flesh firmness was determined in two opposite regions of the equatorial portion of the fruit, with a small portion of the epidermis previously removed using a motorized penetrometer (Güss Manufacturing Ltd., Cape Town, South Africa) equipped with an 11-mm diameter tip and expressing the results in Newton (N);

- The iodine-starch index was determined by reacting the starch with a solution of 12 g of metallic iodine and 24 g of potassium iodide in 1 L of distilled water. The iodine solution was applied to a cut in the peduncular half of the equatorial region of the fruit, after which the color was compared (reaction of iodine with starch) considering a scale of 1-5, in which index 1 indicates the maximum starch content, and index 5 represents the fully hydrolyzed starch (Amarante et al. 2010);
- TA was determined in a 5-mL sample of fruit juice previously extracted from transversal slices taken from the equatorial region of the apples and chopped in an electric centrifuge. The chopped sample was diluted in 45 mL of distilled water, and titrated with 0.1 N sodium hydroxide solution until reaching pH 8.1, using an automatic titrator (Titro Line Easy®, Mainz, Germany). The results are expressed as % malic acid;
- The SS content was determined using a digital refractometer (Atago PR 201α, Tokyo, Japan), with results expressed in °Brix;
- The SS/TA ratio was calculated based on the values obtained for SS and TA.

## Determination of the functional compounds

### Obtaining the extracts

The extracts used to quantify the TPC and TAA in the fruit skin were obtained according to the methodology of Larrauri et al. (1997) modified by Rufino et al. (2007a). In centrifuge tubes (Falcon), 5 g of sample was weighed, 10 mL of methanol water solution (50:50, v/v) was added, and the mixture was homogenized with an ultra-turrax (SilentCruscher M, Heidolph, Germany) and kept at rest for 60 min at room temperature. The samples were centrifuged for 15 min at 4°C, and the supernatant was transferred to a 2-mL volumetric flask. Subsequently, 10 mL of acetone water solution (70:30, v/v) was added to the residue of the first extraction, followed by the extraction procedure above until obtaining the supernatant. Finally, the second supernatant was added to the first one, and the volume of the volumetric flask was adjusted to 25 mL with distilled water.

## Determination of the total antioxidant activity

The TAA was determined using methodologies based on the ability of the extract to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals and 2,2-azinobis (3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) radicals (Rufino et al. 2007a, Rufino et al. 2007b).

The DPPH method consisted of a radical prepared on the day of analysis by diluting DPPH (0.06 mM) in methanol. An aliquot of 0.1 mL of the extract and 3.9 mL of the DPPH solution was transferred to test tubes in triplicate. The mixture was homogenized, and the absorbance was quantified at a wavelength ( $\lambda$ ) of 515 nm 30 min after the beginning of the reaction with the addition of the extract. The calibration curve was generated with standard Trolox solutions, and the results were expressed in antioxidant capacity equivalent to Trolox per 100 g of fresh mass ( $\mu\text{mol Trolox}\cdot 100\text{ g}^{-1}\text{ FW}$ ).

The ABTS method consisted of a radical solution prepared using an ABTS stock solution (7 mM) with potassium persulfate (140 mM), which was kept for 16 h at 20°C. Before the analysis, the radical solution was diluted with ethyl alcohol to an absorbance of  $0.70 \pm 0.05$  at  $\lambda$  734 nm. Aliquots of 30  $\mu\text{L}$  were transferred to Falcon tubes containing 3 mL of the ABTS radical in triplicate, which were then homogenized. After 6 min of reaction, the absorbance was quantified at  $\lambda$  734 nm. The calibration curve was established using standard Trolox solutions, with the results expressed in  $\mu\text{mol Trolox}\cdot 100\text{ g}^{-1}\text{ FW}$ .

## Determination of the total phenolic compounds

The TPC was determined by modifying the methodology described by Roesler et al. (2007). For this purpose, 2.5 mL of Folin-Ciocalteu water solution (30:70 v/v) and 0.5 mL of the extract were added to a tube (Falcon). The tube was shaken in vortex (Gehaka, AV-2, São Paulo, Brazil) and rested for 3 min. At the end of this period, 2 mL of a solution of sodium

carbonate (10%) was added to the tube and homogenized. The reaction time was 1 hour, after which the absorbance of each sample was determined at  $\lambda$  765 nm. The calibration curve was established using gallic acid, and the results were expressed in mg of gallic acid equivalent per 100 g of fresh weight (mg EAG-100 g<sup>-1</sup> FW).

## Determination of the total content of anthocyanins

The total content of anthocyanins in the skin of the apples was determined according to the methodology of Fuleki and Francis (1968), with modifications. In tubes (Falcon), 2.5 g of plant tissue and 15 mL of solvent [ethanol (95%) and hydrochloric acid (1.5 N) (85:15, v/v)] were homogenized with ultra-turrax (SilentCruscher M, Heidolph, Germany) for approximately 1 min and stored for 34 hours (3-4°C). After incubation, the samples were centrifuged at 12,000 rpm for 15 min at 4°C. Later, 2 mL of the supernatant was transferred to a graduated tube, and the volume was adjusted to 50 mL with the extracting solvent. Analyses were performed at  $\lambda$  535 and 700 nm, and the results were expressed in mg of cyanidin-3-glycoside per 100 g of fresh weight (mg cyanidin-3-glycoside-100 g<sup>-1</sup> FW).

The data expressed as percentages were transformed into  $\arcsin \sqrt{x}/100$  and subjected to analysis of variance, and the interaction between treatments and crop season were also analyzed. When the treatment effect was significant, the treatment means were compared using the least significant difference test ( $p < 0.05$ ) in each crop season in which there was a significant interaction or with average data from the crop seasons evaluated when there was no interaction.

## RESULTS AND DISCUSSION

There was no interaction between treatments and crop seasons regarding iodine-starch index, SS content, and flesh firmness (Table 1). Summer pruning did not affect the iodine-starch index or SS content (Table 1). Contrary to the results obtained in this work, Robinson et al. (1983) reported greater starch degradation in apples exposed to a higher light intensity and attributed an increased fruit metabolism to this effect. İkinci (2014), in turn, observed an inconsistent effect of summer pruning on the SS content in peaches, with alternating results throughout the evaluated seasons.

**Table 1.** Iodine-starch index, soluble solids, and flesh firmness of Fuji apples, at harvest, due to the time of summer pruning, in three crop seasons (2016/2017, 2017/2018, and 2018/2019). Mean data for three seasons.

Treatments	Iodine-starch index (1-5)	Soluble solids (°Brix)	Flesh firmness (N)
Without summer pruning	4.5 a*	12.8 a	71.7 bc
Pruning in December	4.6 a	12.5 a	73.1 ab
Pruning in January	4.6 a	12.8 a	70.4 c
Pruning in February	4.7 a	12.6 a	74.0 a
CV (%)	4.3	3.8	3.4

\*Means followed by the same letter in the columns do not differ by the least significant difference test ( $p < 0.05$ ); CV: coefficient of variation.

Summer pruning in February provided greater flesh firmness at harvest than when pruning in January (Table 1). The relationship between light incidence and flesh firmness is highly variable. Studies have shown positive relationships between light incidence and flesh firmness in Fuji apples (Jung and Choi 2010). However, other studies have indicated that apples exposed to a higher light intensity tend to present less flesh firmness due to starch breakdown and increased respiratory metabolism (Robinson et al. 1983), which was not proven by this present study. According to Saure (1987), flesh firmness is a maturation attribute with variable behavior when subjected to summer pruning. However, in the present study, the effect was consistent throughout the three crop seasons evaluated.

The values of TA and the SS/TA ratio varied between crop seasons in response to the treatments applied (Table 2). TA was higher in apples harvested from plants of the control treatment in the 2016/2017 crop season and in fruit of plants submitted

to summer pruning in February in the 2017/2018 crop season. However, there was no difference between treatments for TA in the 2018/2019 crop season. Summer pruning also showed an inconsistent effect of TA for different peach cultivars, which varied according to the studied year (Ikinci 2014, Ikinci et al. 2014). However, the results are not conclusive, and they stated that there are studies that demonstrate that pruning increases the TA, those that have indicated the opposite effect, and others in which no effect was observed (Saure 1987). The influence of summer pruning on this attribute possibly depends on climatic conditions during fruit development.

**Table 2.** Titratable acidity, soluble solids/titratable acidity ratio (SS/TA), and skin background color ( $h^\circ$ ) in Fuji apples, at harvest, due to the time of summer pruning, in three crop seasons (2016/2017, 2017/2018, and 2018/2019).

Treatments	Titrateable acidity (% malic acid)	SS/TA	Skin background color ( $h^\circ$ )
<b>2016/2017</b>			
Without summer pruning	0.46 a*	271 c	101.5 a
Pruning in December	0.38 b	31.6 bc	104.8 a
Pruning in January	0.32 c	38.7 a	92.0 b
Pruning in February	0.34 bc	34.8 ab	98.7 ab
CV (%)	10.7	9.9	5.0
<b>2017/2018</b>			
Without summer pruning	0.35 b	41.6 a	108.4 a
Pruning in December	0.37 b	38.0 a	110.0 a
Pruning in January	0.36 b	39.3 a	108.9 a
Pruning in February	0.44 a	31.8 b	107.8 a
CV (%)	7.3	8.2	1.6
<b>2018/2019</b>			
Without summer pruning	0.39 a	30.3 a	107.1 a
Pruning in December	0.36 a	32.5 a	108.1 a
Pruning in January	0.37 a	32.9 a	104.2 b
Pruning in February	0.39 a	31.0 a	105.2 b
CV (%)	7.1	8.2	1.1

\*Means followed by the same letter in the columns, for the same crop season, do not differ by the least significant difference test ( $p < 0.05$ ); CV: coefficient of variation.

The SS/TA ratio was higher in fruit of plants submitted to summer pruning in January, presenting no significant difference from plants submitted to summer pruning in February in the 2016/2017 crop season. The 2017/2018 crop season showed the lowest SS/TA ratio in the fruits of plants submitted to summer pruning in February, with no difference between treatments in the 2018/2019 crop season. The results of the SS/TA ratio were determined by the variation in TA (Table 2), since the SS values did not differ between treatments (Table 1).

The values of skin background color ( $h^\circ$  of the least red region) varied between crop seasons in response to the treatments applied (Table 2). The skin background color was less green (lower  $h^\circ$  value) in fruit of plants submitted to summer pruning in January in the 2016/2017 crop season, showing no difference from those pruned in February of the same season and in January and February of the 2018/2019 crop season. However, there was no difference between treatments in the 2017/2018 season (Table 2). The final color of apples develops during the ripening process, in which chlorophyll degradation occurs in addition to the synthesis of secondary compounds (Musacchi and Serra 2018). The base color is considered a reliable indicator for evaluating apple ripeness (Saure 1987), for which the lowest  $h^\circ$  of the base color obtained with summer pruning in the two crop seasons studied could indicate early fruit ripening. However, other maturation attributes, such as flesh firmness, SS content, and iodine-starch index, do not support this early maturation with summer pruning, regardless of the season.

There was no interaction between treatments and crop seasons for the development of red color at harvest. Summer pruning in January and February generally provided fruit with a more intense red color (lower  $h^\circ$  value in the redder

region) and a higher proportion of fruit with > 50% of the surface-colored red (Table 3). Even plants pruned in December produced more fruit with this condition compared to plants without summer pruning. The red color of apples is an important factor in encouraging consumer purchase (Stanger et al. 2017). The plants pruned in February produced 37.6% more fruit with > 50% red color than the plants in the control treatment, which resulted in fruit with higher commercial value.

**Table 3.** Fruit coloring in the redder region ( $h^\circ$ ) and percentage of fruit with more than 50% of red coloring, at harvest, due to the time of summer pruning, in three crop seasons (2016/2017, 2017/2018, and 2018/2019). Mean data for three seasons.

Treatments	Redder region ( $h^\circ$ )	Fruit with more than 50% of red coloring (%)
Without summer pruning	51.6 a*	40.5 c
Pruning in December	52.8 a	51.6 b
Pruning in January	41.8 b	56.8 ab
Pruning in February	44.3 b	64.9 a
CV (%)	13.1	22.2

\*Means followed by the same letter in the column do not differ by the least significant difference test ( $p < 0.05$ ); CV: coefficient of variation.

The red color in the skin of apples is the result of anthocyanin synthesis (Bae et al. 2006), which highly depends on light exposure (Jakopic et al. 2009). The content of anthocyanins was normally higher in fruit from plants submitted to summer pruning in January and February in the 2016/2017 and 2017/2018 crop seasons (Table 4). High-light intensity increases the synthesis of flavonoids, especially of the anthocyanin class, in some stages of fruit development, such as during ripening and harvesting (Zoratti et al. 2015). Therefore, pruning in January and February allowed for additional light to penetrate the canopy, improving fruit color. The control plants, for which no side branches or branches that shaded the fruit were removed, showed reduced light passage in the canopy, restricting the synthesis of anthocyanin pigments in the fruit. According to Ashraf and Ashraf (2014), summer pruning can improve light availability to fruit to develop a red color. Although the resumption of vegetative growth after pruning was not evaluated, it was possible to observe that plants pruned in December resumed their vegetative growth during the following months. However, in some cases, coloration can also be impaired since summer pruning can stimulate the growth of other vegetative buds, emitting new branches that shade the fruit (Schupp and Ferree 1988, Saure 1990), what may have occurred in this work for plants pruned in December.

**Table 4.** Content of total anthocyanins (TAN), total phenolic compounds (TPC), and total antioxidant activity (TAA; ABTS and DPPH methods) in the skin of Fuji apples, at harvest, due to the time of the summer pruning, in two crop seasons (2016/2017 and 2017/2018).

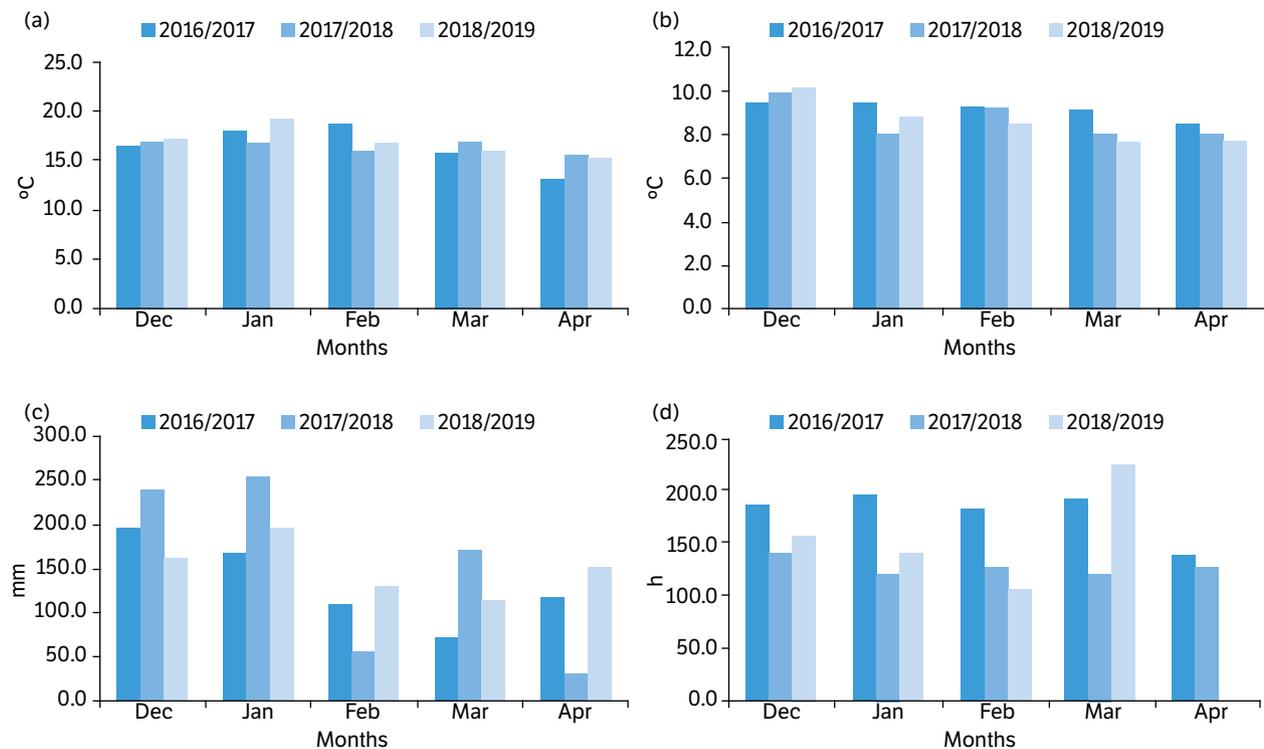
Treatments	TAN (mg cyanidin 3-glycoside·100 g <sup>-1</sup> FW)	TPC (mg EAG·100 g <sup>-1</sup> FW)	TAA ( $\mu$ Mol Trolox·100 g <sup>-1</sup> FW)	
			ABTS	DPPH
<b>2016/2017</b>				
Without summer pruning	33.7 bc*	349.8 a	7,528.0 a	6,856.0 a
Pruning in December	24.4 c	363.0 a	7,367.0 a	9,214.0 a
Pruning in January	57.6 a	383.8 a	8,712.0 a	8,543.0 a
Pruning in February	42.5 ab	402.7 a	6,945.0 a	7,431.0 a
CV (%)	19.4	11.8	21.7	31.8
<b>2017/2018</b>				
Without summer pruning	14.3 bc	253.4 b	3,350.0 ab	5,601.7 b
Pruning in December	13.5 c	224.4 b	2,844.4 b	5,643.3 b
Pruning in January	19.0 ab	370.1 a	3,198.2 ab	8,097.5 a
Pruning in February	25.1 a	438.7 a	3,977.8 a	8,089.2 a
CV (%)	12.6	15.2	11.4	15.0

\*Means followed by the same letter in the columns, for the same crop season, do not differ by the least significant difference test ( $p < 0.05$ ); CV: coefficient of variation; ABTS: 2,2-azinobis (3-ethylbenzothiazoline)-6-sulfonic acid; DPPH: 2,2-diphenyl-1-picrylhydrazyl.

The TPC and TAA values varied between crop seasons in response to the treatments applied (Table 4). The 2016/2017 crop season showed no effect of the treatments on the TPC and TAA values (ABTS and DPPH methods) of the fruit skin. However, the plants pruned in January and February of the 2017/2018 crop season produced fruit with relatively high values of TPC and TAA (evaluated using the DPPH method) (Table 4). TPCs are influenced by environmental factors, especially light (Jakopic et al. 2009). Thus, as occurred with the accumulation of anthocyanins, summer pruning carried out in January and February possibly allowed for additional light to enter the canopy, resulting in higher TPC and TAA values in the fruit skin.

Polyphenols are the primary antioxidant compounds of many fruits and vegetables (Coklar and Akbulut 2017). The main groups of phenolic compounds in apples are phenolic acids, flavonoids, and anthocyanins (Ceymann et al. 2012, Jakobek et al. 2013). This explains the higher content of TAA (DPPH method) in the skin of the fruit of plants submitted to summer pruning in January and February, when higher values of TPC and anthocyanins were also observed.

In the 2016/2017 crop season, in which the fruit was harvested at the end of April (the longest maturation period), the thermal amplitude was higher, the rainfall was less frequent, and there was a higher number of hours of sunshine (Fig. 1). These factors contribute to the accumulation of color in the fruit, which may explain the lack of effect of summer pruning on phenolic compounds and antioxidant activity, as well as the higher content of these variables during this crop season than during the other crop seasons. The climatic data of the 2017/2018 crop season indicate that the opposite occurred in the previous season, when there was a lower average temperature, less thermal amplitude, less sunshine, and, in some months, higher rainfall. These conditions are unfavorable for the accumulation of color in the fruit, and therefore the effect of summer pruning was more evident in this crop season, indicating differences in the content of antioxidants and phenolic compounds.



**Figure 1.** Climatic data on (A) average temperature, (B) thermal amplitude, (C) rainfall, and (D) insolation from the São Joaquim meteorological station (Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina, 2020).

The performance of summer pruning, evaluated after storage and compared to the control treatment (without summer pruning), did not influence the incidence of rot, yellowing of the fruit, flesh firmness, TA, SS content, and SS/TA ratio, regardless of the season (data not presented).

The optimal time for summer pruning is important considering its strong influence on the quality of the fruit produced. The results obtained show that summer pruning in January and February of Fuji Standard apple trees (a clone with fruit of reduced red coloring), with high vigor (orchards implanted several years ago on Marubakaido rootstock with M9 filter), may be a viable alternative to improve the quality of fruit at harvest, especially in harvests unfavorable to the development of the red color. As noted, the benefits from performing this practice at the appropriate time are not only in visual quality, but also in the functional quality of the fruit.

## CONCLUSION

Summer pruning of Fuji Standard apple trees in January and February promoted the development of red coloration and increased the values of phenolic compounds and antioxidant activity in the fruit skin. Furthermore, summer pruning in February resulted in fruit with greater flesh firmness at harvest than that of fruit in the control group. On the other hand, summer pruning in December was ineffective for improving the color and functional properties of the fruit. Finally, summer pruning did not influence the quality of Fuji Standard apples in cold storage.

## AUTHORS' CONTRIBUTION

**Conceptualization:** Lugaresi, A.; **Methodology:** Steffens, C. A., Brighenti, A. F., Pasa, M. S., and Martin, M. S.; **Project administration:** Steffens, C. A., Brighenti, A. F., Pasa, M. S., and Martin, M. S.; **Data curation:** Lugaresi, A., Souza, M. P.; **Funding acquisition:** Steffens, C. A., Amarante, C. V. T., Brighenti, A. F., Pasa, M. S., and Martin, M. S.; **Formal analysis:** Lugaresi, A.; **Investigation:** Lugaresi, A., Steffens, C. A., Souza, M. P., Brighenti, A. F., Pasa, M. S., and Martin, M. S.; **Supervision:** Steffens, C. A.; **Writing – original draft:** Lugaresi, A.; **Writing – review & editing:** Steffens, C. A., Souza, M. P., Amarante, C. V. T., Brighenti, A. F., Pasa, M. S., and Martin, M. S.

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Data will be available upon request.

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