



## Bud position influence the fruit quality attributes of three blueberry (*Vaccinium ashei* Reade) cultivars<sup>1</sup>

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### ABSTRACT

Blueberry farming can be considered an excellent alternative to diversify the family income. In addition, the fruits have several health benefits thanks to the presence of phenolic compounds and anthocyanins, however blueberries chemical composition is affected by edaphoclimatic conditions and bud position within the shoot. Therefore, the objective of the study was to evaluate the influence of the bud position on the total phenolic content, anthocyanins, antioxidant capacity, and other quality parameters of blueberry fruits of three cultivars (Climax, Bluegem, and Powderblue) grown in the mesoregion of Pelotas, Brazil. Fruits of these cultivars were harvested from three shoot position. The fruits were evaluated for their total soluble solids content, pH, total titratable acidity, total soluble solids/total titratable acidity ratio, fruit color, phenolic compounds, anthocyanins, and antioxidant potential against the 2,2-diphenyl-1-picryl-hydrazyl radical. The bud position in the shoots influenced the physicochemical parameters of the blueberry fruits of all cultivars. Fruits harvested from apical levels had higher content of soluble solids, pH, phenolic compounds, anthocyanins, and antioxidant potential, demonstrating that studies aiming at evaluating fruits from different shoot levels are important, as they directly impact the quality of the fruits as well as their biological potentials.

**Keywords:** climax; bluegem; powderblue; bud position; phytochemical.

### INTRODUCTION

Fruit farming is an activity of great importance in Brazil, with the country being the third largest fruit producer in the world, behind only China and India. Among the fruits, blueberry fruits (*Vaccinium* sp.) have caught the attention of consumers, the food industry, marketing agents and farmers (Fachinello 2008; Lima *et al.* 2010; FAO, 2019).

Blueberry farming can be considered an excellent alternative to diversify the family's incomes, as it provides high economic return in small areas of cultivation and in a short period of time. In addition, the cultivation of blueberries requires a low supply of inputs, given its rusticity, a fact that can contribute to the insertion of this crop in agroecological productive models. Moreover, blueberries have a great importance in the food security

of the family nucleus (Oliveira *et al.*, 2020; Marangon & Biasi 2013).

Blueberries are considered a “superfood” due to their rich composition in bioactive compounds, such as phenolic substances and anthocyanins, which have several health benefits, such as antimicrobial, antioxidant, anti-tumor, antihyperglycemic, cardioprotective and neuroprotective activities (Basu *et al.* 2010; Rodrigues *et al.* 2011; You *et al.* 2011; Pertuzatti *et al.* 2016; Wu *et al.* 2017; Hoskin *et al.*, 2019; Wood *et al.*, 2019).

Despite the numerous health benefits, the chemical composition of fruits, such as blueberries, can be influenced by the edaphoclimatic conditions of the region where it is grown, season, leaf density, and the position of the fruits in the shoot (Embrapa, 2010). Therefore, the

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objective of the present study was to evaluate the influence of the bud position on the total phenolic content, anthocyanins, antioxidant capacity, and other quality parameters of fruits of three blueberry fruits of three cultivars (Climax, Bluegem and Powderblue).

## MATERIAL AND METHODS

### *Plant material*

Blueberry plants (*Vaccinium* sp.) Group "Rabbiteye" from the cultivars Climax, Bluegem and Powderblue, grown in an organic commercial orchard located in Morro Redondo, RS (31°32'S 52°34'W, 150 meters high) were used to conduct the experiment during the 2012-2013 harvest.

The plants used for each blueberry cultivar in the Rabbiteye group were eight years old at the time of collection and were in full production. For this purpose, ten experimental units were delimited, with three plants in each unit. The plants were randomly selected in the orchard, three for each cultivar. From each of these 10 branches were selected, with a apical, median, and basal bud in each branch. The plants had long branches between 31 and 50 cm in length, and short branches between 15 and 30 cm in length. Apical fruits were collected from the tip furthest from the base, the medians were collected from the center of each branch and the basal ones were collected close to the base of the branch. The number of fruits, per bud, was evaluated at harvest time, with each branch being one repetition, which totaled 30 repetitions, per cultivar.

For the evaluation of the physicochemical parameters, the fruits were collected, stored in thermal boxes, and transported to the Fruits and Vegetables Laboratory of the Federal University of Pelotas. The analyzes were performed in triplicate.

### *Physicochemical assessments*

The harvested fruits were evaluated as for their total solids soluble content, total titratable acidity, pH, fruit color, phenolic compounds, anthocyanins and antioxidant potential against the DPPH radical.

Total soluble solids content (TSS) was determined by refractometer (Quimis model Q-109B) and expressed in °Brix. The determination of total titratable acidity (TTA) was performed by titration with 0.1 M NaOH solution until the pH stabilized at 8.1 and expressed as a percentage of citric acid according to the AOAC method (2001). The pH was determined using a potentiometer (New Technical Ind. And Com. Brazil). The fruit color evaluation was performed using a colorimeter (Minolta) and was expressed in Hue Angle calculated using the formula:

$$\text{Hue Angle} = \left[ \tan^{-1} \times \frac{b^*}{a^*} \right]$$

Where  $a^*$  defines the green/red scale and  $b^*$  defines the yellow/blue scale. Color measurements were performed on opposite faces in the equatorial region of the fruits.

The content of total phenolic compounds was determined by the method used by Pereira et al. (2013), where the absorbance reading was performed on a spectrophotometer at a wavelength of 765nm and the results expressed in mg of gallic acid 100 g<sup>-1</sup> on a dry basis. The total anthocyanin content was determined by the spectrophotometric method adapted from Lees & Francis (1972), and the absorbance reading was performed on a spectrophotometer at a wavelength of 520nm. The anthocyanin content was expressed in mg of cyanidin-3-glycoside 100 g<sup>-1</sup> of dry matter.

The antioxidant potential was determined using the method established by Rutz et al. (2012), which measures the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) free radical inhibition capacity (2,2-diphenyl-1-picryl-hydrazyl) and the results were expressed in µmol eq. Trolox 100 g<sup>-1</sup> of dry matter.

### *Statistical analysis*

All data were submitted to analysis of variance (p < 0.05) and, when significantly different, the means were compared by the Tukey's HSD statistical method (p < 0.05) using the software Statistica (Supplementary Table 1).

## RESULTS AND DISCUSSION

### *Total soluble solids*

The contents of total soluble solids for the three cultivars at the different shoot levels can be seen in table 1. A statistical difference can be observed between the shoot levels for the cultivars Climax and Powderblue with the highest sugar content, in both cultivars, for the fruits harvested from the apical level. For the Bluegem cultivar, a statistical difference was observed only for the fruits present at the medial level, as these presented the highest content of soluble solids.

The fruits of the cultivar Climax harvested from the medial and basal shoot levels showed similar values of soluble solids, and those fruits harvested at the apical level showed higher values than those reported by Pertuzatti et al. (2016), who found values between 14.4 to 16.4 °Brix for blueberries (cultivar Climax) harvested (without comparison of levels) in the mesoregion of Porto Alegre, Brazil in the 2010-2011 and 2011-2012 harvest seasons, respectively. For the cultivar Bluegem, the study performed by Pertuzatti et al. (2016) found values similar to those reported in our study. Finally, for the cultivar Powderblue, the contents of soluble solids were higher than those reported by Pertuzatti et al. (2016). In addition, the content of soluble solids found for the three cultivars, in our study, were higher than those observed by Saftner

**Supplementary Table 1.** Analysis of variance of blueberry fruits of the cultivars Climax, Bluegem, and Powderblue located in different shoot levels (apical, medial, and basal), grown in the municipality of Morro Redondo, RS in the 2012/2013 harvest season**Total soluble solids**

	<b>GL</b>	<b>SQ</b>	<b>QM</b>	<b>Fc</b>	<b>Pr&gt;Fc</b>
Cultivars	2	7.192	3.5959	154.11	4.7406e-12
Bud position	2	13.059	6.5293	279.82	2.7700e-14
Cultivars * bud position	4	15.448	3.8620	165.52	6.2200e-14
Residue	18	0.420	0.0233		
Total	26	36.119			
		CV: 0.96%			

**Total titrable acidity**

	<b>GL</b>	<b>SQ</b>	<b>QM</b>	<b>Fc</b>	<b>Pr&gt;Fc</b>
Cultivars	2	0.40627	0.203137	18282.3	0.000e+00
Bud position	2	0.14783	0.073915	6652.3	1.500e-26
Cultivars * bud position	4	0.08059	0.020148	1813.3	3.483e-23
Residue	18	0.00020	0.000011		
Total	26	0.63490			
		CV: 0.67%			

**pH**

	<b>GL</b>	<b>SQ</b>	<b>QM</b>	<b>Fc</b>	<b>Pr&gt;Fc</b>
Cultivars	2	0.55814	0.279070	105.976	0.00000000
Bud position	2	0.12699	0.063493	24.111	0.00000810
Cultivars * bud position	4	0.08028	0.020070	7.622	0.00089177
Residue	18	0.04740	0.002633		
Total	26	0.81281			
		CV: 1.59%			

**Total titrable acidity**

	<b>GL</b>	<b>SQ</b>	<b>QM</b>	<b>Fc</b>	<b>Pr&gt;Fc</b>
Cultivars	2	2110.9	1055.45	3545.0	4.3000e-24
Bud position	2	1358.3	679.17	2281.2	2.2350e-22
Cultivars * bud position	4	1037.8	259.45	871.4	2.4813e-20
Residue	18	5.4	0.30		
Total	26	4512.4			
		CV: 1.54%			

**Phenolic compounds**

	<b>GL</b>	<b>SQ</b>	<b>QM</b>	<b>Fc</b>	<b>Pr&gt;Fc</b>
Cultivars	2	548076	274038	20765.9	0.0000e+00
Bud position	2	136044	68022	5154.5	0.0000e+00
Cultivars * bud position	4	26122	6531	494.9	3.8865e-18
Residue	18	238	13		
Total	26	710480			
		CV: 0.25%			

**Antioxidant potencial (DPPH)**

	<b>GL</b>	<b>SQ</b>	<b>QM</b>	<b>Fc</b>	<b>Pr&gt;Fc</b>
Cultivars	2	0.65339	0.32669	630.05	0.0000e+00
Bud position	2	0.05787	0.02894	55.81	1.9200e-08
Cultivars * bud position	4	0.03015	0.00754	14.54	1.8151e-05
Residue	18	0.00933	0.00052		
Total	26	0.75074			
		CV: 1.55%			

Continua...

*Continuação***Anthocyanins**

	<b>GL</b>	<b>SQ</b>	<b>QM</b>	<b>Fc</b>	<b>Pr&gt;Fc</b>
Cultivars	2	96.535	48.268	13575.3	0.0000e+00
Bud position	2	7.150	3.575	1005.5	0.0000e+00
Cultivars * bud position	4	0.179	0.045	12.6	4.5875e-05
Residue	18	0.064	0.004		
Total	26	103.929			
		CV: 0.55%			

**Hue angle**

	<b>GL</b>	<b>SQ</b>	<b>QM</b>	<b>Fc</b>	<b>Pr&gt;Fc</b>
Cultivars	2	1463.47	731.74	3002.11	0.0000e+00
Bud position	2	434.93	217.46	892.20	0.0000e+00
Cultivars * bud position	4	18.91	4.73	19.39	3.1824e-11
Residue	81	19.74	0.24		
Total	89	1937.05			
		CV: 0.17%			

*et al.* (2008) for cultivars of the Rabbiteye group (11 °Brix on average).

Total soluble solids content is a maturation parameter. For blueberry fruits, values equal to or greater than 15 °Brix are considered indicators of optimal maturation stage. The sugar content is directly related to the incidence of solar radiation on the fruits, where the greater the incidence of solar radiation, the greater the accumulation of sugars (Morrison & Noble, 1990). The buds located in the region near the apical part of the shoot receive higher radiation than buds located in the medial and basal levels, which stimulates a greater accumulation of sugars in the fruits located in this region.

**Total titratable acidity**

The content of soluble solids is negatively correlated with the percentage of acidity, where, as the sugar synthesis increases during maturation, the acidity decreases. The fruits of the cultivar Climax, regardless of the shoot level, presented higher percentages of acidity (Table 1), while the lowest values were found in the cultivar Powderblue. The fruits harvested from the different levels of all cultivars showed a statistical difference between them, with a higher percentage of acidity for the fruits located in the basal level. It was observed that the bud position significantly influenced the acidity.

The percentage of acidity found in the cultivar Climax was lower than that found by Pertuzatti *et al.* (2016) in blueberries grown in the mesoregion of Porto Alegre, Brazil, who found acidity ranging from 0.30 to 0.52%, depending on the harvest season. The same study, when evaluating the acidity for the cultivar Bluegem found similar values (0.27 to 0.52%) than those reported in our study, while the values obtained for the cultivar Powderblue were higher

(0.43 to 0.57%) than the ones found in our study. Rodrigues *et al.* (2011), when analyzing the cultivars Powderblue and Bluegem reported that they had 1.28 and 0.95% acidity, respectively, values that are much higher than those reported in our study.

**pH**

For the cultivars Climax and Powderblue, only the fruits of the apical levels showed a statistical difference for the pH values (Table 1), with higher pH in both cultivars. As for the cultivar Bluegem, no statistical differences were observed between the levels. Evaluating the different cultivars for the fruits harvested from the apical level, we only observed a statistical difference for the cultivar Powderblue, while for the medial and basal levels, all cultivars differed statistically from each other, with the cultivar Powderblue being the one with the highest pH at both levels.

The fruit pH is directly related to its composition, since lower pH values help to retain phenolic compounds and anthocyanins. Thus, it is observed that in all cultivars and at both levels, the pH values were low, which may be an indication of protection of bioactive compounds.

The pH values found for the three cultivars and at all bud levels, were similar to those reported by Pertuzatti *et al.* (2016) for blueberries produced in the mesoregion of Porto Alegre, Brazil.

**Total soluble solids/total titratable acidity (TSS/TTA)**

All cultivars showed statistical differences between the bud levels for the TSS/TTA ratio (total soluble solids/total titratable acidity) (Table 1), with the cultivars Climax and Powderblue having the highest TSS/TTA ratio at the

apical level, and for the cultivar Bluegem at the medial level. Statistical differences were observed between cultivars at all levels.

The relationship between the content of total soluble solids and total titratable acidity is related to the balance between sugars and acids present in the fruit, being used as an important indicator of the maturation point for the fruits. During the maturation period, the TSS/TTA ratio tends to increase, as a consequence of the decrease in acids, due to the hydrolysis of polysaccharides and an increase in sugars, a secondary product of the conversion of organic acids. In all cultivars, the TSS/TTA ratio was higher at the apical level, when compared with the medial and basal levels.

The values of the TSS/TTA ratio found for the three cultivars at all levels were higher than those found for the same cultivars produced in the region Vale do Ribeiro, Paraná, Brazil during three harvest seasons, where researchers found values for the cultivar Climax ranging between 7.1 to 19.5, for the cultivar Bluegem between 7.2 to 19.1 and for the cultivar Powderblue between 8.4 to 20 (Medeiros *et al.* 2018).

### **Phenolic compounds**

The fruits of all levels for all the cultivars differed statistically from each other, with higher contents of

phenolic compounds at the apical level for the three cultivars (Table 2). Comparing the three cultivars for each level, we found a statistical difference, with a higher content of phenolic compounds in the cultivar Powderblue and a lower value in the cultivar Bluegem. The bud position influenced the content of special compounds in the fruits, and the fruits located in the apical shoot level showed a concentration of phenolic compounds significantly higher than the fruits located in the medial and basal levels.

The content of phenolic compounds found for the cultivars Climax, Bluegem and Powderblue were higher than those reported by You *et al.* (2011) evaluating blueberries produced in the United States and by Rodrigues *et al.* (2011) evaluating blueberries from the mesoregion of Porto Alegre, Brazil.

The results found in our study were promising, because they demonstrate a good adaptation of the cultivars Climax, Bluegem and Powderblue to the climatic condition of Pelotas mesoregion, Brazil, and to the proper management of the trees, which promotes a greater accumulation of phenolic compounds that have several biological properties such as antitumor, antioxidant, antihyperglycemic and anti-inflammatory activities (Basu *et al.* 2010; You *et al.* 2011; Sukprasansap *et al.* 2020).

**Table 1.** Total soluble solids, total titratable acidity, pH and total soluble solids/total titratable acidity (TSS/TTA) ratio of blueberry fruits of the cultivars Climax, Bluegem and Powderblue located in different shoot levels (apical, medial and basal), grown in the municipality of Morro Redondo, RS in the 2012/2013 harvest season

#### **Total soluble solids**

Cultivar	Apical	Medial	Basal
Climax	17.50 Ba	15.80 Bb	14.93 Bc
Bluegem	14.67 Cb	16.37 Aa	14.93 Bb
Powderblue	18.27 Aa	16.00 Bb	15.47 Ac

#### **Total titratable acidity**

Cultivar	Apical	Medial	Basal
Climax	0.49 Ac	0.68 Ab	0.83 Aa
Bluegem	0.46 Bb	0.43 Bc	0.49 Ba
Powderblue	0.27 Cc	0.40 Cb	0.45 Ca

#### **pH**

Cultivar	Apical	Medial	Basal
Climax	3.17 Ba	3.02 Cb	3.00 Cb
Bluegem	3.19 Ba	3.20 Ba	3.20 Ba
Powderblue	3.58 Aa	3.32 Ab	3.32 Ab

#### **TSS/TTA**

Cultivar	Apical	Medial	Basal
Climax	35.7 Ba	23.2 Cb	18.0 Cc
Bluegem	31.9 Cb	38.1 Ba	30.5 Bc
Powderblue	66.8 Aa	39.7 Ab	34.6 Ac

Different capital letters in the same column statistically differ from each other according to the Tukey's HSD test ( $p \leq 0.05$ ). Different lowercase letters on the same line statistically differ from each other according to the Tukey's HSD test ( $p \leq 0.05$ ).

### **Antioxidant potential against the 2,2-diphenyl-1-picryl-hydrazil (DPPH) radical**

Evaluating the antioxidant potential against the DPPH radical (Table 2) in the cultivars Climax and Powderblue, we observed a statistical difference only for the apical levels, while for the cultivar Bluegem, only the medial level statistically differed from the others. At the apical level, the highest value of inhibition of the DPPH radical was observed in the cultivars Climax and Powderblue, which did not statistically differ from each other. Finally, for the medial and basal levels, we encountered a statistical difference between the cultivars, with the highest inhibition values found in the cultivar Powderblue and the lowest in the cultivar Bluegem.

The fruits harvested in the apical level showed significantly higher antioxidant potential than the antioxidant potential observed for fruits located in the medial and basal levels of the shoots, ensuring that a greater amount of compounds with bioactive properties are produced and accumulated in the fruits of the apical region. The results of the antioxidant potential of the cultivar Climax, at all levels, were higher than those found by Rodrigues *et al.* (2011) in blueberries grown in the mesoregion of Porto Alegre, Brazil, while the cultivars Bluegem and Powderblue showed lower results.

Although the DPPH radical is not produced in the human body, its evaluation is used as a parameter for determining the antioxidant activity of a compound or group of compounds due to its chemical structure. The antioxidant potential of blueberry fruits comes possibly due to the presence of phenolic compounds and anthocyanins that act on the route of the erythroid nuclear factor 2 (Nrf2), which stimulates antioxidant response agents promoting oxidative stress control, controlling several chronic non-communicable diseases (Sukprasansap *et al.* 2020).

### **Anthocyanins**

The cultivar Climax only showed a statistical difference in the anthocyanin content (Table 2) for the apical level, where we observed a higher content. For the cultivars Bluegem and Powderblue, statistical differences were observed between all levels, with Bluegem having greater content of anthocyanins at the medial level and Powderblue at the apical level. Assessing each level in the three cultivars, it is observed that all cultivars statistically differed from each other, with the highest content of anthocyanins in the Powderblue cultivar for all levels and the lowest in the cultivar Bluegem.

**Table 2.** Content of phenolic compounds, anthocyanins, antioxidant potential against the 2,2-diphenyl-1-picryl-hydrazil radical and fruit color (Hue Angle) of blueberry fruits of the cultivars Climax, Bluegem and Powderblue located in different shoot levels (apical, medial and basal), grown in the municipality of Morro Redondo, RS in the 2012/2013 harvest season

#### **Phenolic compounds**

Cultivar	Apical	Medial	Basal
Climax	1553.91 Ba	1355.00 Bc	1362.92 Bb
Bluegem	1362.57 Ca	1147.56 Cc	1258.51 Cb
Powderblue	1664.70 Aa	1586.27 Ab	1564.38 Ac

#### **Antioxidant potential (DPPH)**

Cultivar	Apical	Medial	Basal
Climax	1600Aa	1500 Bb	1500 Bb
Bluegem	1300Ba	1200 Cb	1300 Ca
Powderblue	1700 Aa	1600 Ab	1600 Ab

#### **Anthocyanins**

Cultivar	Apical	Medial	Basal
Climax	61.0 Ba	57.2 Bb	55.6 Bb
Bluegem	43.7 Cb	48.7 Ca	41.1 Cc
Powderblue	68.2 Aa	60.8 Ab	57.6 Ac

#### **Hue angle**

Cultivar	Apical	Medial	Basal
Bluegem	299.03 Aa	294.92 Ab	293.23 Ac
Climax	295.19 Ba	293.30 Bb	290.61 Bc
Powderblue	289.45 Ca	285.10 Cb	283.90 Cc

Different capital letters in the same column statistically differ from each other according to the Tukey's HSD test ( $p \leq 0.05$ ). Different lowercase letters on the same line statistically differ from each other according to the Tukey's HSD test ( $p \leq 0.05$ ).

Anthocyanins are responsible for the coloring of the epidermis and the blueberry pulp. It is believed that the greater accumulation of these compounds in the fruits located in the apical levels may be related to a higher incidence of solar radiation that these fruits receive in relation to the fruits of the medial and basal levels, which are close to the crop canopy and receive less light. According to Riihinens *et al.* (2008), studying European blueberries, fruits located in higher levels are exposed to greater solar radiation and thus accumulate more phenolic compounds and anthocyanins.

In addition to providing color to the fruits, anthocyanins, as well as the phenolic compounds present in blueberries, act in the control of several chronic non-communicable diseases, such as type II diabetes mellitus, cardiovascular diseases, some types of cancers and neurodegenerative diseases (Basu *et al.* 2010; You *et al.* 2011; Sukprasansap *et al.* 2020).

#### **Fruit color (Hue angle)**

The fruit color, measured by the Hue angle (Table 2), statistically differed between the shoot levels of the three cultivars, being in all cases, fruits with greater hue angles located at the apical levels of the shoots. Statistical differences were also observed between cultivars for each level, with the highest hue angle values in the cultivar Bluegem and the lowest in the cultivar Powderblue.

The color of the fruits, indicated by the Hue angle, varied between 283.03 and 299.03, a range of values that indicate the typical bluish color of blueberries. Regarding the bud position on the shoot, it was observed that the fruits present in the apical levels presented a higher hue angle than that observed for the fruits located in the medial and basal levels, which is associated with the greater accumulation of anthocyanins in these fruits, and the highest amount of solar radiation in the upper part of the shoots.

#### **CONCLUSIONS**

The bud position in the shoots influences the physicochemical parameters of the blueberry fruits for the cultivars Climax, Bluegem and Powderblue. Fruits located in the apical levels showed, in general, higher soluble solids, pH, phenolic compounds, anthocyanins and antioxidant potential, thus demonstrating that studies aiming at evaluating fruits at different levels in the shoots are important, as they directly impact the quality of the fruits as well as their biological potentials. These results can contribute, for blueberry producers to test practices to assist in the greater exposure of fruits to solar radiation, as well as in the selection of fruits that will be used for fresh consumption or for industrial processing.

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