

Effects of pruning and season on antioxidant and quality traits of blueberries

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Abstract

Consumer interest in natural antioxidants has risen because their intake is linked to the prevention of chronic diseases. Blueberries (*Vaccinium spp.*)—rich in flavonoids, vitamin C and other bioactive compounds—are therefore being cultivated beyond temperate regions. In tropical areas, however, the lack of chilling hours may alter fruit physiology, and little is known about how pruning practices modulate quality under these conditions. The objective of this study was to quantify the effects of pruning types and seasons on the antioxidant activity, physical and chemical characteristics (TSS, TTA, TSS/TTA), and ash, moisture, and fiber contents in blueberries of the cultivar Biloxi grown under tropical climate conditions (without chilling hours), in Brasília, DF, Brazil. The trial followed a randomized-block design with three replications. Plants received either severe pruning removing lateral branches (SPRLB) or severe pruning maintaining lateral branches (SPMLB) in winter, spring, summer or autumn; fruit harvested over the subsequent four weeks was analysed for DPPH radical-scavenging capacity, total soluble solids (TSS), titratable acidity (TTA), TSS/TTA ratio, ash, moisture and fibre contents. Pruning season exerted the strongest effect. Autumn pruning—regardless of type—produced the highest antioxidant activity and elevated TSS. SPRLB increased TTA, whereas SPMLB enhanced the TSS/TTA ratio during warmer seasons. These findings demonstrate that pruning season plays a key role in enhancing blueberry fruit quality under tropical conditions

Keywords: Biloxi, °Brix, functional properties, nutritional quality, tropical agriculture

Introduction

Blueberry (*Vaccinium spp.*) belongs to the group of deciduous shrub plants known as *Ericaceous Vaccinium*, in the family Ericaceae, and is widely valued for its significant health benefits. Blueberry fruits or blueberries are rich in fibers and have high moisture contents, low caloric value, and high contents of bioactive compounds (Wang *et al.*, 2017). Consuming fruits and vegetables is a proven way to prevent diseases due to the presence of various bioactive compounds (Rufino, 2008), especially antioxidants, which compose a group of compounds that can prevent degenerative processes associated with free radicals in the body (Costa; Rosa, 2006).

Blueberries rank among the five top healthiest foods in the world (Duan *et al.*, 2022). Their use in functional foods has been increasing, as it is a practical and efficient way to raise bioactive compound contents in processed foods for diverse markets. This is evidenced

by the significant increase in blueberry processing worldwide, mainly in the United States, Canada, and China (Duan *et al.*, 2022).

Blueberry juice is one of the most widely consumed blueberry-based products worldwide (Nunes *et al.*, 2019). In addition, blueberries are incorporated into a broad array of commercial foods designed with the purpose of promoting the intake of antioxidants and fibers in diets, including pastries, juices, jellies, wines, yogurts, powdered pulps, dried fruits, jams, and cookies (Cesa *et al.*, 2017; Chaudhary; Verma; Saharan, 2020; Liu *et al.*, 2019; Negi *et al.*, 2022; Perez *et al.*, 2017; Rurush *et al.*, 2022; Sun *et al.*, 2022; Yang *et al.*, 2019).

Pruning is a fundamental technique to regulate vegetative growth and fruit production in blueberries (Strik *et al.*, 2003). Studies conducted in temperate regions have shown that the type of pruning significantly affect yield, fruit maturation and chemical composition

including sugars and acids (De Moura et al., 2017; Jorquera-Fontena et al., 2014). However, few studies have evaluated how these practices influence fruit quality when blueberries are cultivated under tropical conditions, such as in the Cerrado biome of Brazil.

Given the lack of chilling hours and the different temperature regimes in tropical areas (Miranda et al., 2025), it is essential to identify pruning strategies that improve blueberry fruit quality and antioxidant potential. Understanding how different pruning types and seasons affect fruit quality under these conditions is crucial for recommending adequate management practices for tropical blueberry production.

We hypothesize that pruning type and timing exert influence on blueberry fruit quality. Therefore, this study aimed to evaluate the effects of pruning types and seasons on the antioxidant activity and physicochemical characteristics of 'Biloxi' blueberries grown in Brasília, DF, Brazil, under tropical climate conditions without chilling hours.

Materials and methods

Study Area

The study was conducted at the Laboratory of Bioactive Compounds and Nanobiotechnology (LCBNano) of the Institute of Biological Sciences of the University of Brasília (UnB) for antioxidant activity analyses, and at the UnB Fruit Production Laboratory for physical and chemical analyses, both in Brasília, DF, Brazil. Climate

conditions during the experiment are described in **Table 1**.

Samples

The blueberries used for the analyses were collected from an open-field experimental orchard in the Fruit Production Sector at the UnB Experimental Biology Station, Brasília, DF (15°44'11.9"S, 47°52'53.3"W, and altitude of 1010 meters). The region's climate is Aw, according to the Köppen-Geiger classification, characterized by a rainy season in the summer (October to April) and a dry season in the winter (May to September) (Cardoso; Marcuzzo; Barros, 2014).

Fertigation supplied the crop's water and nutrients, delivering—on an annual basis—about 200 kg ha⁻¹ N, 120 kg ha⁻¹ P₂O₅, 250 kg ha⁻¹ K₂O, 200 kg ha⁻¹ Ca²⁺, 120 kg ha⁻¹ Mg²⁺ and 150 kg ha⁻¹ SO₄²⁻, in accordance with fertilization guidelines commonly adopted by local blueberry growers (de Lima et al., 2020).

These blueberry plants were part of an experiment conducted in a randomized block design with 3 replications and 10 plants per plot, totaling 240 plants. The spacing between plants was 1 × 0.4 m. The plants had been subjected to two pruning types: severe pruning removing lateral branches (SPRLB) and severe pruning maintaining lateral branches (SPMLB). Pruning was carried out in all four seasons (winter, spring, summer, and autumn), i.e., at 90-day intervals: August 21 (winter pruning), November 21 (spring pruning), February 21

Table 1. Meteorological data during the experimental period. Monthly mean (Tmean), maximum (Tmax), and minimum (Tmin) temperatures, and monthly number of chilling hours below 18 °C (CH < 18), 15 °C (CH < 15), 12 °C (CH < 12), 10 °C (CH < 10), and 7.2 °C (CH < 7.2), Brasília, DF, Brazil, from August 2019 to February 2021.

Month	Tmean	Tmax	Tmin	CH < 18	CH < 15	CH < 12	CH < 10	CH < 7.2
	°C							
	Hours							
Aug-19	21.4	28.1	15.4	208	51	0	0	0
Sep-19	24.6	31.4	18.5	46	0	0	0	0
Oct-19	24.6	31.3	19.2	26	0	0	0	0
Nov-19	23.2	29.3	18.8	44	0	0	0	0
Dec-19	22.8	28.3	19.0	16	0	0	0	0
Jan-20	22.6	27.6	19.1	14	0	0	0	0
Feb-20	22.1	27.3	18.8	48	0	0	0	0
Mar-20	21.9	27.0	18.6	26	0	0	0	0
Apr-20	21.4	26.7	18.0	65	6	0	0	0
May-20	19.4	25.5	14.5	339	110	30	14	0
Jun-20	19.3	25.6	13.8	342	156	23	1	0
Jul-20	19.3	25.7	13.4	355	148	20	0	0
Aug-20	20.8	27.1	14.4	287	118	14	3	0
Sep-20	23.4	29.8	17.3	97	8	0	0	0
Oct-20	23.8	29.7	19.3	28	0	0	0	0
Nov-20	21.9	27.2	18.1	88	2	0	0	0
Dec-20	22.8	28.4	18.6	49	0	0	0	0
Jan-21	22.4	28.2	18.1	108	1	0	0	0
Feb-21	21.1	26.3	18.4	95	1	0	0	0
Total	-	-	-	2281	601	87	18	0

Source: Brazilian National Institute of Meteorology (INMET, 2023)

(summer pruning), and May 21 (autumn pruning). Freshly harvested fruits were transported to the laboratory and stored in plastic boxes. The harvest dates depended on the pruning type and season.

Harvest And Fruit Extract

Blueberries were weekly harvested from all treatments and stored in a freezer (-20 °C). Weekly harvested fruits were grouped into a lot composed of fruits from four weeks of harvest for extract preparation. Therefore, one extract was obtained for each pruning type and season, composed of blueberries harvested over a month.

The fruits were then subjected to an ultrasound-assisted maceration process for two 30-minute periods, using 200 grams of frozen blueberries in 500 mL of ethanol, with solvent exchange between periods. The extract was obtained using a frequency of 50 kHz and a power of 250 VA, in a water bath heated to 30 °C, for 1 hour, in an ultrasonic bath (Kondentech). The extract was then concentrated in a rotary evaporator (Buchi - R-100) and stored in a freezer at -20 °C. The samples were then weighed and subjected to analyses of antioxidant activity and physical and chemical characteristics.

Antioxidant Activity

Blueberry extracts were evaluated for antioxidant potential through the *in vitro* DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay (Sigma Aldrich), as described by Pires *et al.* (2017), with some modifications. Gallic acid (Dinâmica) was used as standard antioxidant compound. A methanolic DPPH solution (32 µg mL⁻¹) was prepared and 280 µL of this solution was mixed with 20 µL of each fruit extract sample (15 mg mL⁻¹). A solution of DPPH and methanol was used as a negative control, using methanol as a blank; additionally, a blank sample, consisting of each extract + methanol, was also tested, focusing on reducing the possible interference of the solvent.

The test was conducted using three replications of 96-well microplates covered with aluminum foil and incubated at room temperature for 30 minutes. Spectrophotometric readings were taken using a spectrophotometer coupled to a microplate reader at 517 nm (Multiskan FC, Thermo Scientific). Results were expressed as milligrams of gallic acid equivalent per gram of blueberry extract (mg g.a.e. g⁻¹), with the mean absorbance of each sample substituting Y in the standard curve equation ($y = -0.1138x + 0.5549$; $R^2 = 0.9946$). The percentage of DPPH free radical scavenging was obtained through the formula $DPPH\ inhibition\ \% =$

$(AC - AA) / AC * 100$, where AC is the mean absorbance of the DPPH control, and AA is the mean absorbance of each fruit extract.

Physical And Chemical Analyses

Total soluble solids (TSS; °Brix), total titratable acidity (TTA; %), TSS to TTA ratio (TSS/TTA), and contents of ashes (%), fibers (g 100 g⁻¹), and moisture (%) were obtained through laboratory analyses using mature blueberries for each pruning type and season. Chemical analyses were performed according to analytical standards of the Instituto Adolfo Lutz (2008).

Total soluble solids (TSS) were determined using a portable refractometer (HI96801; Hanna Instruments) with scale of 0 to 85 °Brix. The results were expressed in °Brix.

Total titratable acidity (TTA) was determined through titration using an alkaline standard solution for assessing the total fruit acidity (peel and pulp). A standard 0.1 M NaOH solution was prepared, and the solution correction factor was calculated. Fruit samples of each replication and treatment were then prepared, containing 10 g of fruit juice diluted into 50 mL of distilled water. Titration was carried out in a 25-mL burette under agitation, using 3 drops of phenolphthalein as indicator, followed by titration with 0.1 M NaOH (standardized).

TSS/TTA was obtained through the ratio between TSS and TTA found for each plot. This ratio is the best method to assess the quality of a fruit product, as it is used as an indicator of ripeness level.

Ash contents were determined after complete carbonization of the sample in a Bunsen burner and calcinated in a muffle furnace at 550 °C until a white-gray ash was obtained (IAL, 2004).

Fiber contents were determined using the gravimetric method; results were expressed as percentage of crude fiber, following the procedure established by the Instituto Adolfo Lutz (2008).

Moisture contents were determined by drying the samples to a constant weight in an oven at 105 °C, following the methodology described by the Instituto Adolfo Lutz (2008).

Statistical Analyses

The experimental data were subjected to analysis of variance using the F test at a 5% significance level. The means were compared by the Scott-Knott test at a 5% significance level. All statistical analyses and graph development were performed using Sisvar and GraphPad Prism 9.0.

Results and Discussion

Pruning type and season significantly affected the antioxidant potential of blueberries. Considering the effect of pruning types within the pruning seasons, severe pruning removing lateral branches (SPRLB) resulted in higher antioxidant potential when conducted in autumn and spring (Figure 1A). Regarding pruning in winter and summer, SPRLB and severe pruning maintaining lateral branches (SPMLB) resulted in similar antioxidant potentials ($p > 0.05$). The antioxidant potential of blueberries ranged from 289.620 mg g.a.e. g⁻¹ for autumn SPRLB to 212.036 g.a.e. mg⁻¹ for winter SPMLB.

Autumn SPRLB and SPMLB treatments resulted in the highest antioxidant potentials. This finding indicates that fruits from these treatments are more relevant for protecting against oxidative stress and, potentially, more beneficial to human health compared to those from the other treatments.

The percentage of DPPH free radical scavenging was affected by pruning types and seasons. Considering the effect of pruning types within the seasons, SPRLB resulted in a higher percentage than SPMLB in spring (Figure 1B). Regarding the other seasons, the pruning type had no significant effects on this variable ($p > 0.05$).

Pruning season significantly affected DPPH free radical scavenging. Autumn SPRLB and SPMLB treatments resulted in higher percentages (Figure 1B), showing greater antioxidant activity in blueberries from plants pruned in autumn compared to fruits from plants pruned in the other seasons.

Pruning type had a significant effect on TSS. Overall, SPMLB yielded fruits with higher TSS compared to SPRLB. However, these differences were significant only in spring and autumn treatments. In winter, SPRLB produced fruits with higher TSS compared to SPMLB (Figure 2A).

Pruning season had a significant effect on fruit TSS.

Regarding SPRLB, autumn pruning resulted in a lower TSS content compared to pruning in the other seasons (Figure 2A). For SPMLB, the highest TSS contents were found for autumn and the lowest for winter pruning. These results highlight the importance of pruning season for producing high-quality blueberries.

The effect of pruning type within pruning seasons was significant for fruit TTA. SPRLB yielded fruits with higher TTA when conducted in winter, summer, and autumn (Figure 2B), whereas SPMLB resulted in higher TTA only in spring. SPMLB and SPRLB resulted in significantly different fruit acidity. For SPRLB, the highest TTA was found for winter and the lowest for summer pruning. For SPMLB, the highest and lowest mean TTA values were found for spring and summer pruning treatments, respectively.

Pruning type significantly affected TSS to TTA ratio (TSS/TTA) only in summer and autumn, with SPMLB presenting the highest TSS/TTA for these two seasons (Figure 2C). The pruning type had no significant effect within spring and winter seasons.

The effect of pruning season was significant for all treatments (Figure 2C). Summer pruning resulted in the highest TSS/TTA, regardless of pruning type. Winter and autumn SPRLB resulted in lower TSS/TTA than the other treatments, which were not significantly different from each other ($p > 0.05$). However, winter SPMLB and SPRLB resulted in the lowest mean TSS/TTA values.

Pruning type significantly affected ash contents within pruning seasons. SPRLB resulted in higher ash contents when conducted in summer, autumn, and spring (Figure 3A). SPMLB yielded higher fruit ash contents than SPRLB only when conducted in winter.

Pruning season also significantly affected ash contents. Regarding SPRLB, the highest ash contents were found for summer, followed by autumn, spring, and winter pruning. For SPMLB, the highest ash contents were

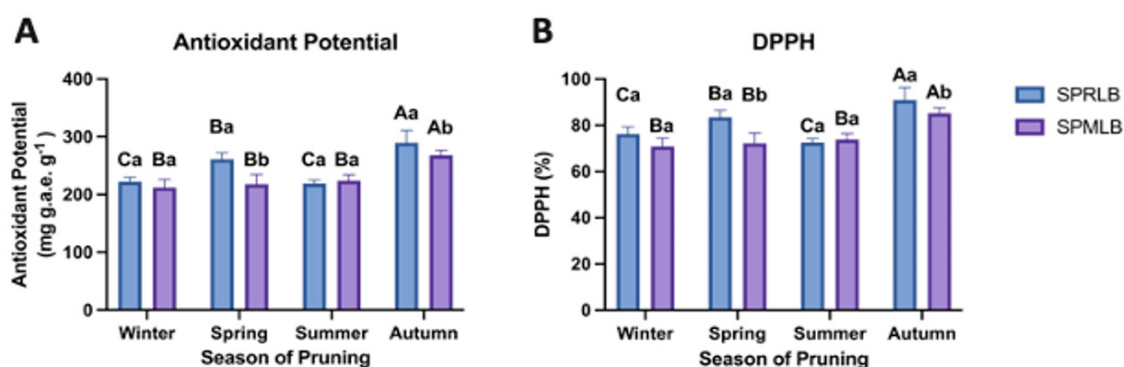


Figure 1. Antioxidant potential and percentage of DPPH free radical scavenging in blueberry extracts as a function of pruning types and seasons.

Bars with different uppercase letters comparing effects of pruning types within seasons, or lowercase letters comparing effects of seasons within pruning types, are different from each other by the Scott-Knott test at a 5% significance level.

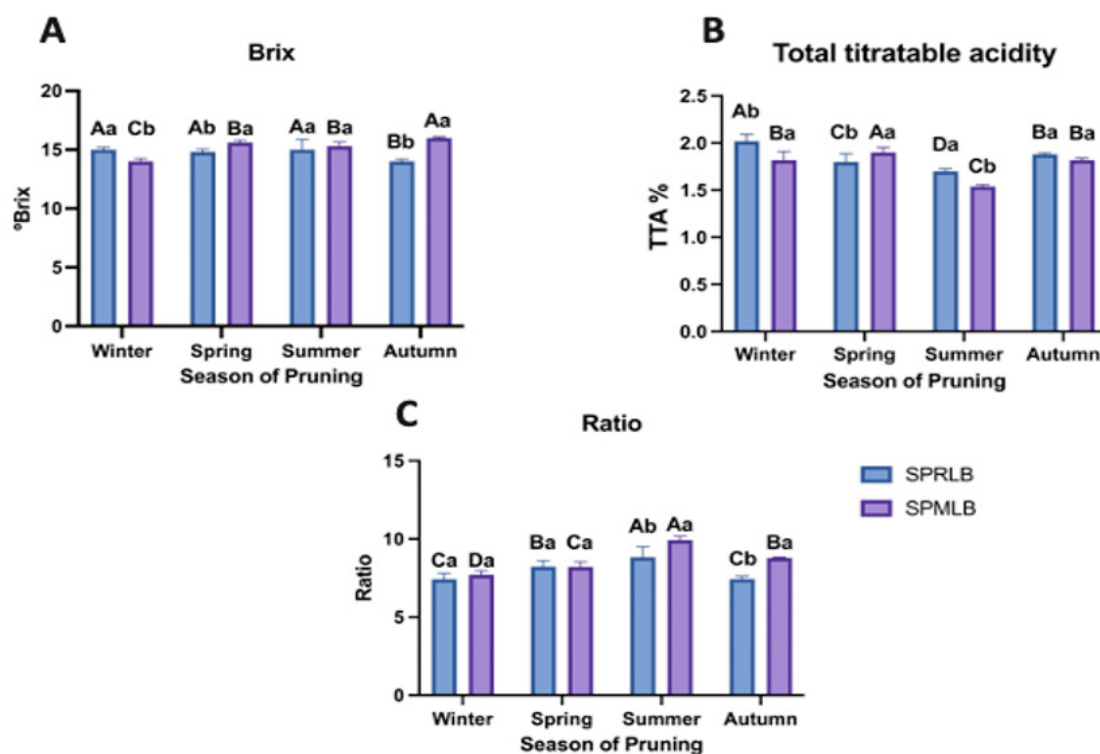


Figure 2. Total soluble solids (TSS; °Brix), total titratable acidity (TTA), and TSS to TTA ratio (TSS/TTA) in blueberry extracts as a function of pruning types and seasons.

Bars with different uppercase letters comparing effects of pruning types within seasons, or lowercase letters comparing effects of seasons within pruning types, are different from each other by the Scott-Knott test at a 5% significance level.

found for winter, followed by summer, autumn, and spring pruning.

The effect of pruning type within the seasons was significant for fruit moisture content. SPRLB resulted in the highest moisture content only when conducted in winter (Figure 3B). In the other seasons, SPMLB resulted in the highest moisture content.

Pruning season had a significant effect on fruit moisture content for both pruning types. Autumn SPRLB and SPMLB treatments resulted in the highest moisture content. The lowest moisture contents were found for summer SPRLB and winter SPMLB treatments.

SPRLB yielded fruits with higher fiber contents than SPMLB in winter (Figure 3C); the opposite was found in summer. Not significant difference in fiber contents was found between both pruning types in spring and autumn.

Pruning season significantly affected fiber contents. For SPRLB, the highest and lowest fiber contents were found in fruits from plants pruned in winter and summer. For SPMLB, the highest fiber contents were found for spring and autumn treatments, and the lowest for summer and winter treatments.

Blueberries are among the most antioxidant-rich fruits commercially grown worldwide and rank among the healthiest fruits due to their nutritional properties. Blueberry crops have expanded to tropical climate

areas due to the development of new low-chill cultivars, significantly increasing the availability of blueberries on the world market and, consequently, their consumption.

However, data on the nutritional quality of blueberries from plants grown in tropical climates (with mean minimum temperatures above 7.2 °C), especially those grown in the Cerrado biome in Brazil, remain scarce. Therefore, the present study assessed the effects of two pruning types conducted in different seasons on the antioxidant activity and physical and chemical characteristics of blueberries grown in Brasília, DF, Brazil.

The highest antioxidant potential of blueberries was found for severe pruning removing lateral branches (SPRLB) carried out in autumn. The antioxidant potential of fruits varied depending on the pruning season. Routray and Orsat (2014) evaluated blueberries of the cultivars Nelson and Elliot harvested in different months and found a 69% variation between the highest and lowest antioxidant activities depending on the harvest timing. Similarly, Radünz *et al.* (2014) evaluated blueberries of three cultivars grown in Pelotas, RS, Brazil, and found variation in antioxidant activity depending on the pruning season. Therefore, the pruning season effect on the antioxidant potential of fruits was more significant than the effect of pruning types practiced in the blueberry orchard.

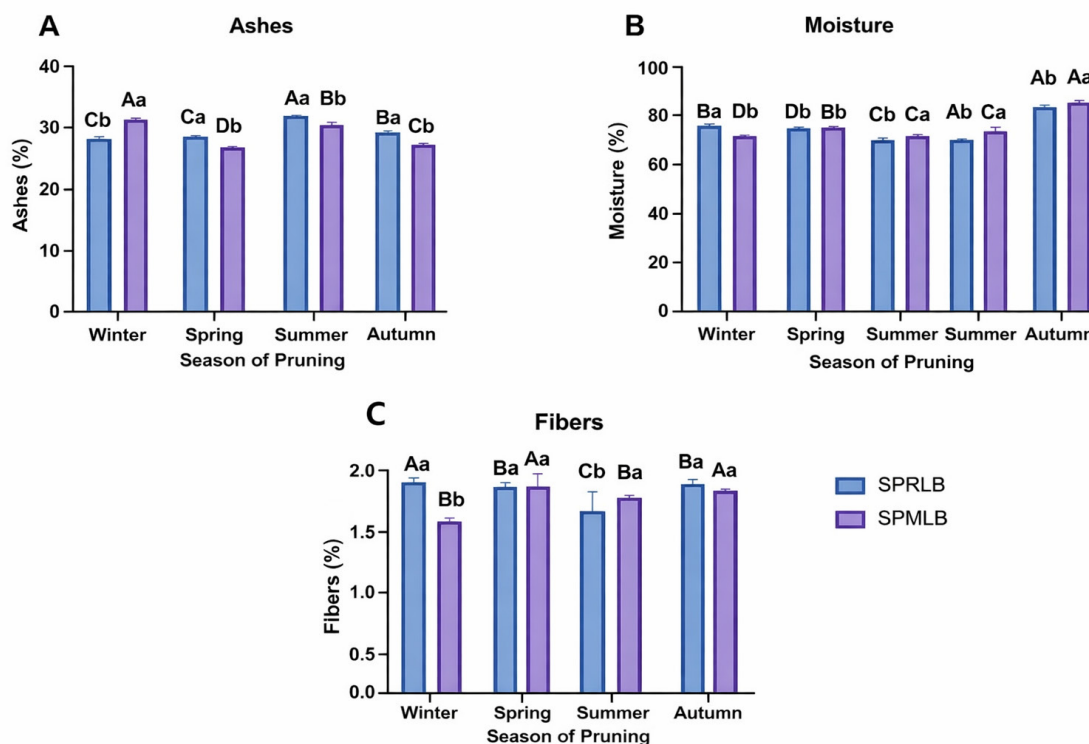


Figure 3. Ash, moisture, and fiber contents in blueberry extracts as a function of pruning types and seasons. Bars with different uppercase letters comparing effects of pruning types within seasons, or lowercase letters comparing effects of seasons within pruning types, are different from each other by the Scott-Knott test at a 5% significance level

Bianchi (2017) evaluated blueberries of the cultivar Climax grown in the South region of Brazil and found higher antioxidant activity in fruits from plants pruned in the coldest month (May 2020). Similarly, Arellanes-Juárez, Benito-Bautista, and Zárate-Nicolás (2023) found more bioactive compounds in blueberries from plants of the cultivar Biloxi pruned during low-temperature periods in the Oaxaca region, Mexico. However, other factors affect the availability of antioxidants in fruits, denoting the need for further studies evaluating the availability of these compounds when the fruit is ready for consumption. Moreover, the experiments conducted in the present study can be replicated in different months of the year or to test 30-day pruning intervals for assessing the harvest feasibility and mean temperatures during harvest months.

The mean TSS values found in the present work ranged from 16 °Brix for severe pruning maintaining lateral branches (SPMLB) in autumn to 14 °Brix for autumn and winter SPRLB treatments. These means were higher than those found by Arellanes-Juárez, Benito-Bautista, and Zárate-Nicolás (2023) for Biloxi blueberries grown in Oaxaca, Mexico, as they found a range from 11.9 to 13.6 °Brix for fruits harvested within 4 months (July-October). Furthermore, Frías-Ortega *et al.* (2020) assessed the response of Biloxi blueberries to different nutrient concentrations in the irrigation solution and found TSS ranging from 10.6 to 13.2 °Brix, lower than those found in

the present study.

TSS (°Brix) is an essential quality factor for blueberries, as higher °Brix indicates a higher sugar content in the fruit. The higher TSS values found in the present work, compared to those reported in the literature, indicate fruits with high quality and flavor. However, flavor is a complex characteristic connected to the amounts of acids and volatiles and the food matrix; thus, establishing a simple correlation between TSS and fruit sweetness is not possible (Cordenunsi *et al.*, 2002).

The range found for TTA (1.54% to 2.02%) was higher than that found by Hera, Sturzeanu, and Teodorescu (2021) (0.12% to 1.25%) when evaluating the quality of fruits of seven blueberry cultivars. Despite the mean TTA values were higher than those reported in other studies on Biloxi blueberry, they are within the range found by Galletta *et al.* (1971), who assessed physical and chemical characteristics of 104 blueberry clones grown in the United States.

However, the mean TSS/TTA values found in the present study were lower than those reported in other studies (Fang *et al.*, 2020; Medeiros *et al.*, 2021). Additionally, these TTA means indicate that fruits grown in the Cerrado biome in Distrito Federal, Brazil, tend to have a sourer flavor (Galletta *et al.*, 1971) due to their higher TTA.

Moisture contents in blueberries varied from

67.6% (winter SPMLB) to 81.7% (autumn SPMLB), while ash contents varied from 26.3% (spring SPRLB) to 32.1% (winter SPMLB). Rocha Concenço *et al.* (2014) found a higher moisture content (87.5%) and lower ash content (17%) in blueberries of the cultivar Bluegem grown in the Southeast region of Brazil. The results found for physical and chemical characteristics in the present study indicate high-quality fruits based on parameters established by Saftner *et al.* (2008).

Fruit fiber contents varied from 1.52% (winter SPMLB) to 1.94% (winter SPRLB). Lameiro *et al.* (2019) evaluated physical and chemical parameters in blueberries grown in the South region of Brazil and found higher fiber contents. Nevertheless, the mean fiber contents found in the present study are consistent with those reported in the literature, denoting the fruit potential to improve human health and decrease the risk of cardiovascular diseases (Sinha *et al.*, 2012).

Conclusion

The results found in the present work corroborate the evidence that blueberries have functional qualities as a food due to its high antioxidant potential.

The pruning of blueberry plants in autumn yielded fruits with higher antioxidant potential and higher total soluble solids contents. Additionally, fruits in all treatments presented satisfactory total soluble solids contents, with some variations depending on the pruning type and season.

Blueberries grown in Brasília, DF, Brazil, under tropical climate conditions, without chilling hours (temperatures below 7.2 °C), presented satisfactory physical and chemical characteristics, meeting the market demands. Therefore, pruning blueberry plants in autumn is recommended for obtaining fruits with higher antioxidant activity.

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