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RESEARCH ARTICLE

Anatomical determinants of fruit firmness in blueberries: A tissue-level analysis

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ABSTRACT

Fruit firmness is an important quality characteristic in blueberries (*Vaccinium corymbosum* L.) because it determines the sensory quality of fresh fruit, its postharvest shelf-life, its ability for long-distance transport, and the feasibility of mechanical harvest. Fruit firmness is genetically determined, but several factors, including the time of harvest, Ca nutrition, pre- and postharvest ambient temperature, and other management practices, also affect gene expression. Little is known about the effect of the anatomy of the different fruit tissues on their firmness. We investigated the anatomy of the different tissues of the fruit in three blueberry cultivars with contrasting firmness: 'Jewell' with 146.6 ± 29.2 g mm^{-1} mechanical compression firmness; 'Flicker' with 185.2 ± 16.6 g mm^{-1}, and 'Sweetcrisp' with 210 ± 62.1 g mm^{-1}. The tissues analyzed were the epidermis, hypodermis, outer mesocarp, and middle mesocarp of fruits collected at harvest time. These tissues were then fixed, embedded in paraffin, stained, sectioned, and mounted for microscopic observation. Statistical differences were found among cultivars concerning epidermal cell size, with 'Jewell' having the largest epidermal cell size and 'Sweetcrisp' the smallest. 'Flicker' showed larger hypodermal cells than 'Jewell' and 'Sweetcrisp'. 'Jewell' presented smaller cells in the outer mesocarp when compared to 'Flicker' and 'Sweetcrisp'. 'Jewel' had no sclereids in the hypodermal layers, while 'Flicker' and 'Sweetcrisp' exhibited high degrees of sclerification. The higher degree of sclerification observed below the epidermal tissue was directly associated with increased firmness. Firm fruit cultivars possess anatomic differences compared to soft fruit cultivars, suggesting an intimate relation between anatomical traits and fruit firmness.

Key words: Anatomical traits, blueberry, fruit firmness, *Vaccinium corymbosum*.

INTRODUCTION

Cultivated blueberries are native to North America and belong to the genus *Vaccinium* in the Ericaceae family. There are about 450 species in this genus, but just a few have been domesticated. Species of economic importance include the highbush blueberry (*V. corymbosum* L.), the rabbiteye blueberry (*V. ashei* J.M. Reade), and the lowbush blueberry (*V. angustifolium* Aiton) (Neugebauer et al., 2024).

Of the so-called small fruits, highbush blueberries are the most cultivated worldwide because of an exponential increase in human consumption due to their sensory quality and the health-promoting bioactive molecules found in their fruit (Radmann et al., 2023).

Blueberries are consumed year-round in most countries of the Northern Hemisphere, so trade between the Southern and Northern hemispheres is increasing (Piennar, 2018). In the context of long-distance transport, fruit firmness is a crucial attribute that determines fruit quality at the point of commercialization (Binghua et al., 2019).

Fruit firmness is commonly defined as the fruit's resistance to a force applied to its surface. This characteristic affects not only the fruit's capacity for long-distance transport but also the consumer's preference, the sensory

quality for fresh consumption, its postharvest shelf-life, and the feasibility of fruit mechanical harvesting (Acevedo, 2014; Cappai et al., 2018). A closely related trait to firmness is crispiness or crunchiness, which stands out for producing extra-firm fruit that further enhances consumer preference (Cappai et al., 2018).

Fruit firmness is genetically determined and variability exists among different cultivars. Several factors influence the genetic expression of this trait, such as the stage of fruit maturity, Ca nutrition, and preharvest and postharvest management of the fruit. For instance, during the fruit development period, high or low temperatures are critical factors that affect fruit quality in general and fruit firmness in particular (Cappai et al., 2018; Olmedo et al., 2021). Soil aeration, irrigation frequency, and irrigation length are also critical factors (Ortega-Farias et al., 2021). The rapid cooling of the fruit after harvest, combined with low ambient temperatures and high relative humidity during selection, packing, storage, and transport, are factors to consider (Kumar et al., 2018).

On the other hand, fruit softening or the loss of fruit firmness is a phenomenon associated with the onset of ripening, which is affected by various fruit characteristics like the thickness of the cuticle, the type and amount of wax deposited on top of it, which is commonly referred as “bloom”; and the size of the fruit scar remaining after removal of the fruit pedicel, all of which affect fruit water loss from the fruit (Cappai et al., 2018; Olmedo et al., 2021; Rivera et al., 2022).

Fruit firmness is also affected by the structure of the cell tissues of the fruit, which vary depending on the type of flower they originate from. The cell layers of fruits can be derived either entirely from the ovary or from both the ovary and the floral cup, resulting in different cuticle thicknesses, structures, and functionalities (Salazar-Duque et al., 2021).

Cell wall integrity plays a significant role in softening most fruits, so its disassembly or the loss of cell wall components is directly responsible for this occurrence (Chen et al., 2015; Olmedo et al., 2021). These components mainly correspond to hemicelluloses, cellulose, and pectin in the primary wall and middle lamella (Chea et al., 2020). Here, Ca and B are crucial for forming pectic bonds that provide mechanical strength to the cell wall (Montecchiarini et al., 2021). Research on blueberries shows increased pectin solubilization and a decrease in hemicellulose and cellulose content of fruits during ripening is concordant with a loss of firmness (Chen et al., 2015; Olmedo et al., 2021). The secondary wall of lignified sclereids also influences firmness. Sclereids can strengthen cell tissue, depending on their quantity and distribution, thereby increasing fruit firmness (Cappai et al., 2018).

Studies on the complexities of cell wall and middle lamella degradation during fruit growth and ripening reveal that this process is mediated by enzymes that catalyze degradation (Kraemer et al., 2021). These include the activities of enzymes such as polygalacturonase, which is related to pectin breakdown and cell wall dissolution; cellulase, which degrades cellulose and specific components of hemicelluloses; and β-galactosidase and glycosidases, all of which are linked to cell wall degradation (Chen et al., 2015).

As shown, firmness has been studied from various multifactorial aspects. However, little attention has been paid to the anatomy of the fruit tissues and how they can affect fruit firmness. The few published anatomical studies focus on the most external cell layers, overlooking the fact that fruits are integral structures affected by the outermost cell layers and the entire fruit matrix. The intricate relation between fruit firmness and its anatomical characteristics appears crucial for understanding fruit quality, marketability, and consumer acceptance. As exposed above, fruit firmness is a genetically determined trait, and even under different environmental conditions, no evidence suggests that a cultivar classified as firm, such as ‘Sweetcrisp’, can become soft to the point of resembling a low-firmness cultivar like ‘Jewell’.

Based on the previous information, we hypothesize that the anatomical traits of blueberry cultivars with high firmness differ from those with lower firmness.

To do this, we studied the anatomy of the structural components of the fruit tissues of three blueberry cultivars with contrasting firmness and compared them to determine if there was a relation between anatomy and firmness. In addition to the descriptive insights provided by anatomical analyses, a statistical analysis focused on cell size was included. Statistical analyses in anatomy remain a challenging subject due to the lack of consensus on the optimal approach to integrate quantitative and qualitative data (Balduzzi et al., 2017).

Therefore, this study aims to determine whether anatomical differences among blueberry cultivars explain variation in their fruit firmness.

MATERIALS AND METHODS

Plant material

Three Southern Highbush blueberry (*Vaccinium corymbosum* L.) cultivars with contrasting firmness, as determined by our own measurements, were used: 'Jewel' with 146 ± 29 g mm^{-1} mechanical compression firmness; 'Flicker' with 185 ± 17 g mm^{-1}; and 'Sweetcrisp' with 210 ± 62 g mm^{-1}.

Experimental procedure

Plants growing in 30-L air-pot containers, spaced 0.30 m apart within a row and 1 m between rows, were used in the experiment. We pruned, irrigated, fertilized, and controlled pests and diseases following standard agronomic procedures to ensure optimal growing conditions. At harvest time (December), nine fruits per cultivar were randomly collected by hand from available bushes when most fruits had turned entirely blue (maturity index for blueberries). Fruits were harvested early in the morning and then bagged and placed in an ice-refrigerated container. Fruit firmness was measured using a texturometer (HappyVolt FirmPro, La Reina, Chile).

Anatomical studies

At harvest time, 24 additional fruits were randomly collected from each cultivar and stored in 50 mL jars containing a 70% ethanol solution. Processing of the samples started almost immediately after the collection of the fruits to produce paraffin-mounted samples for light microscopy observation. We used 24 fruits per cultivar for this analysis. Stored samples were rinsed in deionized water and then dehydrated in a graded series of ethanol (85°, 90°, and 100° for 12 h each, followed by two additional 100° treatments for 3 h). Then, through a series of ethanol-Neo-Clear (Merck KGaA, Darmstadt, Germany), a xylene substitute solution, for 3 h each (97.5°-2.5°, 95°-5°, 85°-15°, 75°-25°, 50°-50°, 25°-75°, 0°-100°, and 0°-100°) and embedded in paraffin following the modified Johansen (1940) histological paraffin technique. They were then sectioned at 15 µm in a ultramicrotome (1416, Leitz, Oberkochen, Germany) provided with stainless steel knives. Sections were stained with a battery of different stains, including tannic acid, ferric chloride, safranin, and fast-green, to be visualized under a light microscope (BA310, Motic, Richmond, British Columbia, Canada). A total of 24 slides per cultivar were used for microscopical observation. Cell size was measured on the epidermis, hypodermis, outer mesocarp, and middle mesocarp tissues using the free-hand method in Image J software (Schneider et al., 2012). These sections were divided into three concentric rows of vascular bundles as described by Cano-Medrano and Darnell (1997). For each tissue, a minimum of 10 cells per slide were measured within a 0.25 mm^2 area. Cell arrangement, cell-to-cell contact, intercellular spaces, sclerification degree, and other characteristics of the parenchymatic tissue were described.

Since no standard method for sclereid counting was available, we used a 3-level scale to estimate the density of sclereids in the fruit flesh. Level 1 corresponds to "no sclereids present"; Level 2, "moderately-sclerified tissue"; and Level 3, "highly sclerified tissue".

Statistical analysis

An ANOVA followed by Tukey's test was used to determine significant differences ($p < 0.05$) in the analysis of epidermal cell area as the data met the assumptions of normality and homoscedasticity. For the rest of the parameters, the Kruskal-Wallis's test was performed, followed by Dunn's test to determine significant differences ($p < 0.05$) between cultivars. The InfoStat software (nfoStat Group, Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Argentina) was used for all statistical analyses.

RESULTS AND DISCUSSION

General anatomy of the blueberry fruit

Blueberry fruit is a berry originated from a flower with an inferior ovary. The outermost layer of the fruit is the epidermis, and underneath it lies the hypodermis. These two cellular tissues derived from the ovary and parts of the calyx, and together form the exocarp, which exhibits a dark color due to a cuticle deposited on the epidermis surface and the thickened primary wall of the hypodermis. The mesocarp, consisting of an outer,

middle, and inner layer, is formed of parenchymal cells in most fruits. The innermost layer is the endocarp, recognizable by its elongated and highly sclerified cells that encase the locules. The seeds are located within these locules, surrounded by the endocarp. This general description aligns with the information presented in Figure 1.

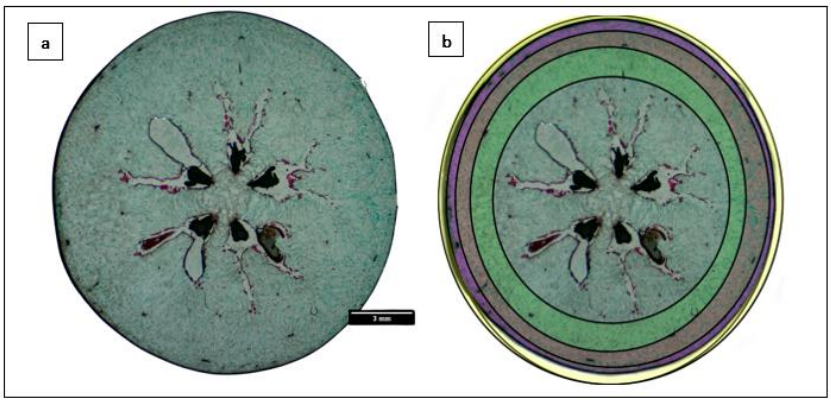

Figure 1. Equatorial section of a 'Jewel' blueberry ripe fruit showing the histological tissues observed with an optical magnifying glass. (a) General anatomy of blueberry fruit. (b) Approximation of the subdivisions of the fruit's cell layers from the outside inward: Epidermis (yellow), hypodermis (pink), outer mesocarp (orange), and middle mesocarp (green). The innermost cell layer is the endocarp that surrounds the seed cavities.

Epidermis

The epidermis is the external cell layer of the fruit, composed of a single row of cells. Figure 2 shows the epidermis of 'Jewel´ (a, b), 'Flicker' (c, d), and 'Sweetcrisp' (e, f). These cells have thickened cell walls covered by an irregularly deposited cuticle composed of epicuticular waxes and cutin (Evert and Eichhorn, 2012). Epidermal cells are rectangular or square-shaped, compact, and contain abundant pigments. These pigments correspond mainly to anthocyanins, which are responsible for the antioxidant properties of blueberries (Krishna et al., 2023). We consistently observed these characteristics in the three cultivars under evaluation (Figure 2).

Epidermal cell size ranged 551-660 μm^2, with 'Jewel' having 660 ± 167 μm^2, the largest epidermal cell size, while 'Flicker' and 'Sweetcrisp' having 612 ± 142 μm^2 and 551 ± 104 μm^2, respectively (Table 1). These values fall within the ranges reported for blueberries in previous studies (Blaker and Olmstead, 2014). According to Table 1, the epidermis displayed significant differences in cell area among cultivars. As shown, 'Sweetcrisp' had smaller cells than 'Jewell' ($p < 0.05$).

This information contradicts Blaker and Olmstead's (2014) work, which reports no relation between epidermal cell size and fruit firmness in blueberries. However, studies conducted on tomatoes report an inverse relationship between fruit firmness and epidermal cell size (Vela et al., 2017), as observed in this investigation, where 'Sweetcrisp' possesses smaller cells, which aligns with greater firmness. It is important to note that tomato fruit develops from a superior ovary (Naika et al., 2005), different from blueberries, which indicates that the epidermal cell size effect on firmness not only depends on the species but also on the ontogeny of the fruit (Cappai et al., 2018).

Several studies suggest the importance of the epidermis in fruit firmness, particularly the structural characteristics of the outermost layers that are responsible for the generation of the cuticle that covers the fruit, which plays an important role in fruit dehydration, the main cause of firmness loss (Paniagua et al., 2013; Rivera et al., 2022; Park et al., 2023).

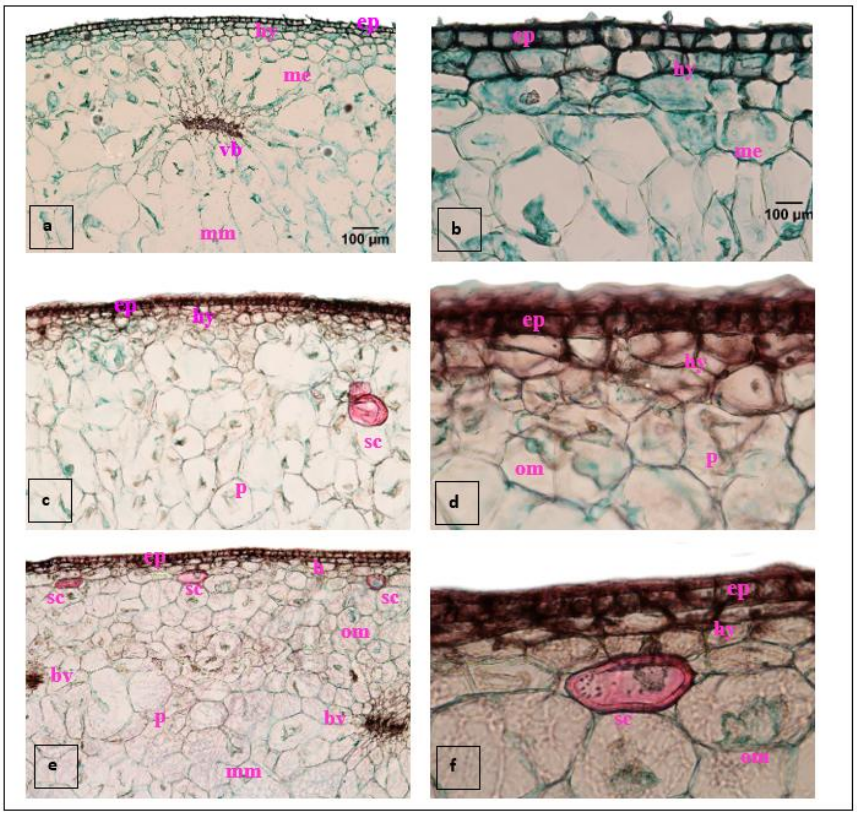

Figure 2. Histological sections showing epidermis in 'Jewel' (a, b), 'Flicker' (c, d), and 'Sweetcrisp' blueberries (e, f). Anatomical similarities are evident among the three cultivars, particularly in the cell layers, where conglomerates of cells surround the vascular bundles. ep: Epidermis; hy: hypodermis; om: outer mesocarp; mm: middle mesocarp; sc: sclereids; vb: vascular bundles.

Table 1. Mean cell size of the epidermis, hypodermis, external mesocarp, and middle mesocarp in three Southern Highbush blueberry cultivars with contrasting firmness. Different letters in the vertical direction imply significant differences ($p < 0.05$).

	Tissue			
Cultivar	Epidermis	Hypodermis	Outer mesocarp	Middle mesocarp
	$\mu m^2 \pm sd$			
Jewel	660 ± 167^{a}	950 ± 299^{a}	5 105 ± 2 271^{a}	12 861 ± 2 516
Flicker	612 ± 142^{ab}	1 747 ± 1 210^{b}	7 520 ± 2 463^{b}	14 379 ± 3 347
Sweetcrisp	551 ± 104^{b}	1 129 ± 434^{a}	7 750 ± 3 102^{b}	15 022 ± 4 720
Tests used:	ANOVA, Tukey,	Kruskal-Wallis, Dunn	Kruskal-Wallis, Dunn	-

Hypodermis

The hypodermis comprises 1, 2, or 3 rows of polyhedral, rectangular, and compact cells, which are generally larger than the epidermal cells and are typically found in the collenchyma. The hypodermal cells of all three cultivars share common features, as they possess thickened walls, with or without pigments, and are generally larger than the epidermal cells. This same observation has also been documented by Cano-Medrano and Darnell (1997) and Fava et al. (2006). Similarities observed between the blueberry epidermis and hypodermis probably result from both cell tissues coming from the hypanthium (Yang et al., 2021). The hypodermal cell wall

is primarily composed of hydrated cellulose, with hemicellulose and sometimes pectin. The primary function of these cells is to provide structural support; therefore, hypodermis cells could play a fundamental role in fruit firmness (Crang et al., 2018).

The size of the hypodermal cells varied between 950 ± 299 and 1747 ± 1210 μm^2, with 'Jewel' showing the smallest size and 'Flicker' the highest. 'Sweetcrisp' exhibited an intermediate value of 1129 ± 434 μm^2 (Table 1). Notably, 'Flicker' had a significantly larger cell area than 'Jewel' ($p < 0.05$), suggesting a significant relation between firmness and larger cells in the hypodermis. Nonetheless, nonsignificant differences were observed between 'Jewell' and 'Sweetcrisp'. The values presented here are like those reported previously for blueberry fruits (Blaker and Olmstead, 2014).

Blaker and Olmstead (2014) proposed that blueberry cultivars with firm fruits possess smaller cells in the outer fruit layers than those with soft fruits. However, in our study, 'Jewel' had smaller cells than 'Flicker' and 'Sweetcrisp' but was less firm.

Within this framework, cultivar-dependent differences in cell wall properties, such as cell wall thickness and composition, may have a critical effect on tissue mechanical resistance beyond cell size alone. In addition, the 3D arrangement of cells, cell packing density, and intercellular connectivity may substantially influence fruit firmness (Majda et al., 2022), indicating that this trait emerges from the interaction of multiple structural and biochemical attributes instead of a single anatomical parameter.

As mentioned in the description of the epidermis, the outermost fruit tissues play a significant role in firmness due to their exposure to the environment, primarily because of their involvement in the dehydration process (Park et al., 2023).

Mesocarp (outer, middle, and internal)

The mesocarp is located between the hypodermis and the endocarp (Figure 1). This tissue consists of polyhedral parenchymatic cells with thin walls that may or may not exhibit pigmentation. In all three cultivars, we frequently observe intercellular spaces that are not visible in the epidermis or the hypodermis. This cell layer is notably larger than the hypodermis and covers approximately 50% of the volume of the fruit. Within it, three concentric sectors of vascular bundles are arranged, forming cell conglomerates around them (Figure 3).

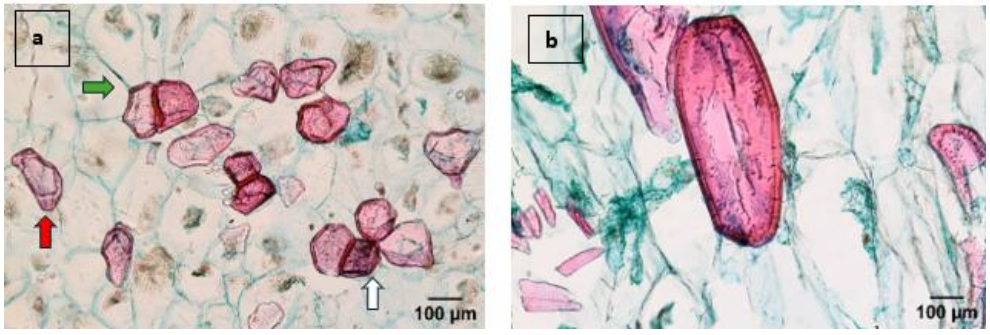

Figure 3. (a) Sclereids in the mesocarp can be found alone (red arrow), in pairs (green arrow), or in groups (white arrow). Cells adjacent to the indicated pair of sclereids form a rosette (10X). (b) Sclereid observed at a microscopic magnification of 40X.

The parenchyma consists of metabolically active living cells that are capable of division and differentiation. An essential function of the parenchyma is photosynthesis, as its cells act as storage tissue, facilitating the assimilation of nutrients, the secretion of various compounds, and respiration (Crang et al., 2018). On occasion, the parenchyma cells can also function as filler tissue, forming the pith and cortex, and therefore are involved in the apoplastic and symplastic transport and storage of different compounds (Crang et al., 2018).

Sclereids are also distributed throughout the mesocarp. Sclereids have thick, highly lignified secondary walls and branched pits and can be found alone, in pairs, or grouped (Figures 3a and 3b). After staining with safranin, they show an intense red color due to the presence of lignin (Figure 3).

Sclerification in blueberry fruits has been described both in highbush cultivars like 'Elliot' (Allan-Wojitas et al., 2001), 'Sweetcrisp' (Blaker and Olmstead, 2014), and 'Coville' (Gough, 1983); and in rabbiteye cultivars like 'Beckyblue' (Cano-Medrano and Darnell, 1997) and most authors relate sclerified tissue in the mesocarp to fruit firmness (Gough, 1983; Cano-Medrano and Darnell, 1997; Fava et al., 2006). Based on these observations, Cappai et al. (2018) hypothesized that this type of cell strengthens the surrounding tissues, contributing positively to increased fruit firmness. However, Blaker and Olmstead (2014) did not find any difference in the number of sclereids present in the different tissues when comparing crisp and non-crisp cultivar fruits. Other authors suggested that the influence of sclereids on firmness could be due to more subtle changes than just the number of sclereids, indicating that the arrangement of parenchyma cells adjacent to the sclereids described as a rosette (Figure 3) can play a significant role in firmness (Allan-Wojitas et al., 2001). From a mechanical perspective, this rosette-like cell architecture may contribute to tissue reinforcement by limiting the deformation of surrounding tissue through a more homogenous cell shape and distribution, thereby influencing the overall resistance of the mesocarp to deformation. The contribution of sclereids on firmness may also result from their effect on the parenchyma and other surrounding water-filled tissues, conditioning cell turgor, rather than simply from their presence or absence (Zhang et al., 2024). Consequently, sclereids not only affect cell functionality but also form a specific 3D cell arrangement that may contribute to the physical resistance of the different tissues (Majda et al., 2022).

Outer mesocarp

The outer mesocarp is the tissue between the hypodermis and the first large row of vascular bundles (Allan-Wojitas et al., 2001). Blaker and Olmstead (2014) reported that blueberry fruits with large cells in the subepidermal layers are associated with low firmness. In our case, the cell size of the outer mesocarp was 5105 ± 2271 μm^2 for 'Jewel', while for 'Flicker' and 'Sweetcrisp', values were 7520 ± 2463 and 7750 ± 3102 μm^2, respectively (Table 1). 'Jewel' showed significant differences compared to 'Flicker' and 'Sweetcrisp' ($p < 0.05$). This information suggests that fruits with larger cells in the outer mesocarp layers have greater firmness.

Various reports indicate that the outer mesocarp is one of the layers that exhibits the most significant variation in cell size during fruit development. Cell size and number play a substantial role in determining the final size of the fruit, which may be related to fruit firmness (Yang et al., 2021).

Middle mesocarp

The middle mesocarp possesses the same characteristics as the outer mesocarp, differing from the latter in that it has larger cells. This tissue is located between the first and second rows of large vascular bundles present in the fruit, comprising most of the mesocarp.

The cell size of the middle mesocarp was 12 861 ± 2 516 μm^2 for 'Jewel', whereas for 'Flicker' and 'Sweetcrisp', values were 14 379 ± 3 347 and 15 022 ± 4 720 μm^2, respectively (Table 1). Nonsignificant differences were observed in the cell area of the middle mesocarp between cultivars.

As mentioned earlier, the outer and intermediate mesocarp characteristics are almost identical, probably because they both constitute the mesocarp and, therefore, have a common origin, which is the walls of the ovary.

Internal mesocarp

As part of the mesocarp, this layer is composed of parenchymal cells. Although they share various features, this cell layer can be viewed as a transitional layer between the hardened endocarp and the middle mesocarp. During cell measurement and descriptions of the inner mesocarp, we observed that they acquired an "irregular" shape, as if it were a projection from the locules to the water-filled surrounding tissues.

Quantitative cell size measurements were not performed for this layer due to its limited characterization and cellular organization; however, descriptive anatomical analysis provides valuable insight into its potential functional role. The internal mesocarp layer remains understudied in terms of its relationship to overall fruit firmness in most fruits. The limited evidence comes from table grapes, where research has suggested that firm cultivars may differ from softer varieties in their internal mesocarp cell wall monosaccharide composition (Balic et al., 2022). However, as grapes originate from a superior ovary (Moreno-Sanz et al., 2020), the formation and separation of fruit tissue layers differ from that of blueberries. As mentioned, this layer lies between the

hardened endocarp that protects the seeds and the water-filled surrounding tissues; thus, we consider it as a transition layer, highlighting its potential role in mediating tissue firmness.

Endocarp

The innermost layer is the endocarp. This cell layer comprises elongated sclerified cells that surround the locules and delimit the end of the inner mesocarp.

Its hardened structure contains the turgid mesocarp cells, which prevent them from pushing into the seed tissue, allowing locule formation where seeds develop. This interaction may lead to desirable fruit firmness due to the stiffness proportioned by the sclerified tissue mentioned before.

Seeds

Seeds are complex structures within plants that contain everything needed to grow and develop a new, complete individual, conditioned by external conditions. Meristematic cells comprise seeds, coated by a lignified structure (Figure 4).

Notwithstanding, the possible seed contribution to the overall fruit firmness collides with the fact that seed number harms consumer acceptance. Suboptimal numbers or the absence of seeds may also lead to smaller and flavor-altered fruit (Cullen et al., 2024), thereby affecting its marketability.

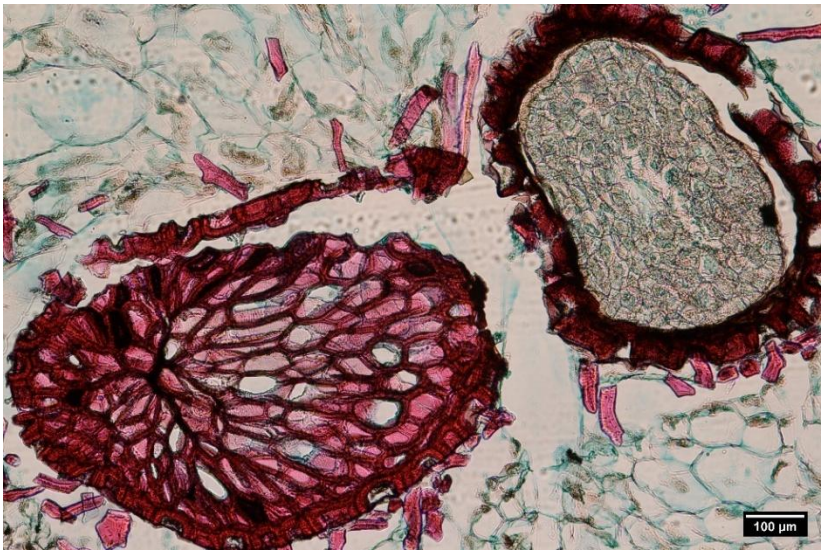

Figure 4. Blueberry seed. Blueish cells inside the lignified structures correspond to metabolically active cells. The seed lignified coating exhibits an intense red color after staining.

Sclerification degree

This three levels scale refers to the sclerified cells along the parenchymatous tissue of the fruit. We proposed this scale because there is a lack of a standard method to evaluate this parameter. As mentioned in the Materials and Methods section, the three levels are defined as follows: Level 1, where no sclereids are present; Level 2 characterized by moderately sclerified tissue; and Level 3 characterized by highly sclerified tissue. 'Jewel' presented 24 out of 24 samples rated as Level 1, as no sclereids were found in the sub-epidermal layers (Figure 5). For 'Flicker', 15 out of 24 samples were evaluated with Level 1, whilst 8 out of 24 presented Level 2, and one sample presented Level 3 (Figure 4).

Regarding 'Sweetcrisp', no samples showed Level 1. While 18 out of 24 samples obtained Level 2, and the remaining six samples presented Level 3 (Figure 5), which suggests that cultivars with greater firmness possess higher sub-epidermal sclerified tissue.

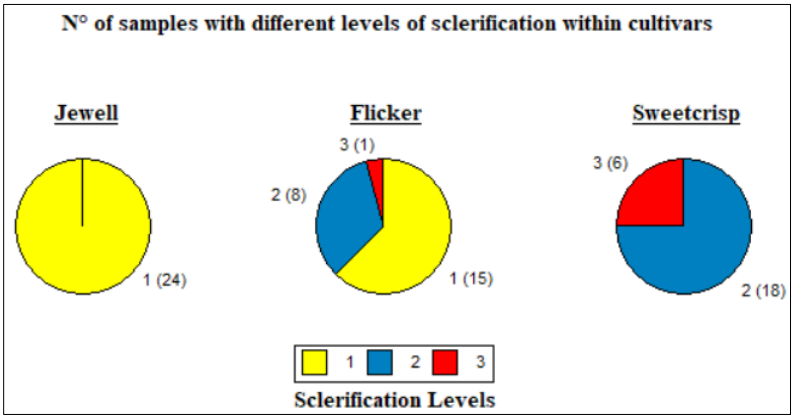

Figure 5. Number of samples with different degrees of sclerification by treatment. Each graph includes a total of 24 samples per treatment. Level 1: Without sclerification; Level 2: moderately sclerified tissue; Level 3: highly sclerified tissue.

Relevant considerations

As mentioned earlier, cell walls play a crucial role in determining fruit firmness. Among several other functions, cell walls provide tensile strength, which is closely related to cell turgor through a sophisticated interaction that defines cell size and shape (Cosgrove, 2022). As cells are filled with water, turgor pressure is crucial for maintaining metabolically active, functional cells; plant growth; cell expansion; and morphogenesis. This phenomenon is generated by intracellular pressure caused by water, where plasma membranes and cell walls play a fundamental role in keeping it in place, much like an inflated balloon with its surface under tension. Although this simplification might be rudimentary, it is helpful to have an idea of the complex interaction of visco-plasto-elasticity of cell walls related to turgor (Zhang et al., 2024).

Regarding cell size, larger cells with a larger radius will likely exhibit lower turgor pressure if cell wall thickness and tensile stress are maintained (Zhang et al., 2024), which could, in turn, imply lower firmness. Moreover, the cell wall content present in fruits can be associated with cell size. If the same cellular surface is considered, and this space is occupied by small cells, it will have a higher cell wall content than one occupied by larger cells. Furthermore, data show that larger cells tend to burst rather than separate (Blaker and Olmstead, 2014), which could imply lower cell wall integrity and cell-to-cell contact, characteristics associated with firmness (Rivera et al., 2022). In a second study by Blaker and Olmstead (2014) on blueberries, no relation was observed between the amount of cell wall material and firm or soft fruit genotypes.

However, this study found that firm-fruit cultivars had larger cells than those of soft fruit. Little evidence backs this up, but Majda et al. (2022) suggested elongated cells, which may be associated with larger cells, could provide anisotropic stiffness due to softer ends in the growing direction but stiffer when stretched longitudinally. Despite the apparent clear relation between these two traits, further research is needed to conclude this.

As discussed throughout this study, cell walls, turgor pressure, and cell size are all interconnected factors that influence fruit firmness. At the tissue level, cell turgor cannot exist without a “contention barrier.” Although cell walls are the main contention barrier, the endocarp and the epidermis serve as the backbone that holds water-filled tissues. This affects tissue stiffness, turgor, and consequently, fruit firmness.

Fruit firmness is affected by anatomical traits from a cellular level, including cell walls, turgor, cell size, and shape. These cellular traits define tissue structure based on cell arrangement, such as the parenchymatous three-dimensional “rosette” arrangement, which sometimes conforms when sclereids are present. From a broader perspective, firmness is influenced by the entire interaction of the seeds, endocarp, mesocarp, hypodermis, and epidermis, where the endocarp and epidermis serve as the pillars for maintaining fruit structure.

CONCLUSIONS

This study highlights anatomical differences between blueberry fruits from firm and soft fruit cultivars. Specifically, fruits from firm cultivars possess larger cell areas in the hypodermis and outer mesocarp, which may contribute to their overall texture. Furthermore, the analysis of 'Jewel', 'Flicker', and 'Sweetcrisp' fruits reveals a direct relationship between the presence of sclerified tissue within the parenchyma of the pericarp and each genotype's fruit firmness. These findings enhance our understanding of the anatomical traits associated with fruit firmness and may provide anatomical targets for breeding programs aimed at improving fruit firmness.

Author contribution

Conceptualization: S.I., L.P., C.M. Methodology: S.I., P.N., L.P., C.M. Formal analysis: S.I., P.N., L.P., C.M. Investigation: S.I. Resources: L.P., C.M. Data curation: L.P. Writing original draft: S.I. Writing review & editing: L.P., C.M. Project administration: L.P. Funding acquisition: L.P., C.M. All coauthors reviewed the final version and approved the manuscript before submission.

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