Evaluation of Cell Wall Modification in Two Strawberry Cultivars with Contrasted Softness

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Abstract: During the ripening process of fruit, the solubilization and depolymerization of cell wall components takes place, which results in the loss of firmness or the softening of fruit. Recently, we reported that two different strawberry cultivars (“Cristal” and “Portola”) exhibit differences in their fruit softening values, with “Cristal” being the firmest and “Portola” being the softest. In the present work, we performed a comparative study of the changes in the physicochemical properties of the cell wall-associated polysaccharide contents of these two strawberry fruit cultivars via thermogravimetric analysis (TGA), combined with the first derivative of the thermogram (DTG) curves and morphological studies using scanning electron microscopy (SEM). The “Cristal” sample showed higher thermal stability than the “Portola” sample. Additionally, differences were observed between the “Cristal” and “Portola” samples at different stages, principally in Region II (temperatures between 200 °C and 350 °C), with a higher thermal stability evident in the green stage of the two cultivars. Notably, a higher thermal stability was observed in the green stage of the “Portola” sample. The highest percentage of cumulative depolymerization (PCD) was observed in the ripe stage of the “Portola” sample. The DTG curve showed four maximum peaks of degradation, which occurred between 170 °C and 350 °C. Finally, the existence of a relationship between fruit firmness and thermal stability was demonstrated for the two cultivars. This relationship was based on the morphological studies conducted using SEM, which provided new evidence through which to understand the changes within the cell wall polymers of these two strawberry cultivars during the ripening process.

Keywords: cell wall disassembly; scanning electron microscopy; strawberry; thermogravimetry analyses

1. Introduction

Commercial strawberry (Fragaria × ananassa Duchesne) is a financially important fruit product that has a short postharvest shelf life due to a rapid softening ratio during ripening [1]. The texture of the fruit is commercially important for consumer acceptability; thus, understanding the textural changes in this fruit is of great interest [1]. The cell walls of fleshy fruits, such as strawberry, show structural and compositional changes during ripening [1–3]. These changes lead to decreased firmness and facilitate the attack of pathogens, increasing postharvest fruit decay and decreasing the quality of fresh fruit [1]. The cell wall is a dynamic and complex supramolecular structure that regulates the development and growth of plants, provides cellular shape and mechanical support, and acts as the first barrier against different biotic and abiotic stresses [4,5]. Its structure is formed by crystalline cellulose microfibrils surrounded by an amorphous matrix of polysaccharides, such as pectins and hemicelluloses, proteins, and inorganic molecules [6–8]. The disassembly of the cell wall structure principally implies the modification of the pectin fractions because pectins are the major components of fruit cell walls [9]. These changes include

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solubilization, depolymerization, and the loss of neutral side chains; other polymers that exhibit changes include hemicelluloses [9]. The disassembly of a cell wall is achieved by the coordinated action of several enzymes that can produce changes in polysaccharide solubilization and depolymerization, and these changes result in fruit softening during fruit development and ripening [10–14]. Thus, softening is partly explained by the breakdown of cell walls [5,11,13–15]. The process of fruit ripening requires a complex set of environmental and endogenous signals [16], and in strawberries and other fleshy fruits and fruit products, these signals produce changes in physiology and morphology, which include fruit softening due to textural changes [2,5,11,15,17–22]. Although these textural changes are important for achieving the maximum sensory quality, they limit the postharvest life of fruit products [13,23]. Independent of the cultivars, strawberry fruits soften quickly during the ripening process [2,11,21]. Different authors have highlighted that the main decrease in fruit firmness occurs between the large green (G) and white (W) stages in strawberry [5,11,13–15].

This process has been investigated using different techniques in most fleshy fruits (including strawberry), but the relative extent and timing can vary among species and even among different cultivars of a given species, leading to different softening rates. We evaluated the fruit firmness of four strawberry types (“Camarosa”, “Cristal”, “Monterey” and “Portola”) grown in the same commercial orchard; notably, “Portola” was the softest cultivar and “Cristal” was the firmest, at the ripe stage [11]. Previously, we performed thermogravimetric analysis (TGA) to evaluate the changes in the physiological properties of the “Camarosa” strawberry fruit, and this cultivar showed a lower thermal stability in the ripe stage than in the green stage [24], and a similar result was obtained from the Chilean strawberry [25].

In the present work, we perform a comparative study of the changes in the physicochemical properties of the cell wall-associated polysaccharides contents of these two strawberry fruit cultivars, grown in the same commercial orchard, via thermogravimetric analysis (TGA) combined with the first derivative of the thermogram (DTG) curves and morphological studies using SEM; furthermore, we investigate the percentage of degradation of the cell wall components.

2. Materials and Methods

2.1. Plant Material

Fruits from *F. × ananassa* “Cristal” and “Portola” were harvested from plants grown in a commercial orchard in Chanco, Maule Region, Chile (latitude 35°50′00″ S; longitude 72°38′00″ W). The harvests were during the 2018–2019 and 2019–2020 seasons. The harvested fruits were immediately transported to the laboratory (Multidisciplinary Agriculture Research Laboratory, Universidad Autónoma de Chile) under cold conditions. For each cultivar and developmental stage, 20 fruits without external damage were analyzed from each season. Firmness was measured using a texture analyzer (model CT3, Brookfield Engineering Labs., Middleborough, MA, USA) with a 1 mm diameter cylinder probe. Each fruit was punctured at the equatorial region on opposite sides (as two technical replicates) and the results were expressed in Newtons (N). The fruits were classified into three different developmental stages (stage 1: green stage; stage 2: 50% ripe or 50% red stage; and stage 3: ripe or 100% red stage) according to Castro and Morales-Quintana, 2019 [24].

2.2. Thermogravimetric Analysis

For thermogravimetric analysis (TGA), the thalami of three different fruits from each sample group were first separated from the achenes. The thalami were homogenized with a mortar and pestle, and the macerated thalami were then dried at 80 °C for 48 h, as according to Castro et al. (2021a; 2021b) [25,26]. These dry samples were ready for the following analysis. One dry sample (10 mg) from the sample pools of the three different stages was employed to evaluate the chemical characteristics of the degradation process by
using a Discovery SDT-650 thermogravimetric analyzer (TA instrument, New Castle 19720, Wilmington, DE, USA), in which the samples were heated in nitrogen at a constant rate of 10 °C min⁻¹ to temperatures of between 50 °C and 550 °C. Additionally, we used Equation (1) to calculate the percentage of cumulative depolymerization (PCD):

\[
\%_{\text{PCD}} = \frac{w_i - w_{\text{max}}}{w_i} \times 100
\]

where \( w_i \) is the initial mass of the sample and \( w_{\text{max}} \) is the mass obtained at the maximum temperature in °C for each peak in the first derivative of the thermogravimetric curves (TG/DTG thermogram), as according to Castro et al. (2021a) [25].

2.3. Scanning Electron Microscopy

Scanning electron microscopy (SEM) was used to examine the morphological changes of the cell wall at different stages in the “Portola” strawberry tissue using the methodology described by Jara et al. (2019) [27]. In short, the thalami of five fruits were homogenized with a mortar and pestle, and the macerated thalami were then dried at 80 °C for 48 h, as according to [27]. The fixed samples were placed directly on copper stubs, air dried at 37 °C for 30 min to 1 h, and gold coated for 3 min at 10⁻¹ Torr with a sputter device (Edwards S150, Sussex, UK). A JSM-6380LV scanning electron microscope (JEOL, Tokyo, Japan) was used with the following settings: accelerating voltage of 15 kV, secondary electron image, and working distance of 15 nm. All the samples were nonconductive and coated in 10 nm of gold using the sputtering technique.

3. Results and Discussion

Firmness was evaluated in two commercial cultivars (“Cristal” and “Portola”) at their ripe stage: “Cristal” was the firmest and “Portola” was the softest from the same seasons (Figure S1). Similarly, “Cristal” and “Portola” showed firmness reduction of 81% and 88%, respectively, from the green stage to the ripe stage; the firmness values were similar to those previously reported for the same cultivars the previous season (2016–2017) [11], indicating that firmness profiles do not change between different seasons, at least during the last 3 years. Additionally, a correlation has been shown between fruit firmness, enzyme activity, and the abundance of mRNA in the main genes (such as polygalacturonase, β-galactosidase, cellulase, expansin and others) involved in the degradation and disassembly of the structural components of strawberry cell walls [11]. However, the changes in the physiological properties of the cell wall-associated polysaccharide contents of these two strawberry fruit cultivars that cause these changes in firmness have not yet been determined. This information is important to understanding the changes in the polymer matrix of the cell wall during the ripening of these two strawberry fruit cultivars.

3.1. Degradation and Thermal Stability Analysis of the Cell Wall in the Two Cultivars at the Three Different Fruit Ripening Stages Using TG/DTG Thermogram Assays

To evaluate the small differences in the integrity, stability and structural organization of the principal polymers that constitute the cell wall during the ripening of the two strawberry fruit cultivars with contrasting softening rates, we measured the thermal stability of thalamus tissues derived from these fruit cultivars at their three main developmental stages. Figure 1 shows three graphics with the TGA curves of “Cristal” (Figure 1A), “Portola” (Figure 1B), or both (Figure 1C). These curves were divided into three phases or regions. The initial phase (Region I) was related to temperatures between 50 and 180 °C, the second phase (Region II) was related to temperatures between 200 °C and 350 °C, and the final phase (Region III) was related to temperatures between 350 and 550 °C, which was independent of the sample stage or cultivar (Figure 1C). Region I showed a slight decrease in mass due to the release of unbound water during the drying procedure [24]. Region II showed a large decrease in the mass percentage (Figure 1A,B), which was caused by the thermal decomposition of hemicellulose and cellulose [24–26,28]. Region III was where the
samples exhibited the thermal decomposition of small concentrations of lignin [24,28–30], but there were differences between the mass losses of the two cultivars. Notably, no significant differences were observed in Region II for “Portola”, while important differences were observed between the green stage and the other two stages of “Cristal” (Figure 1A,B). In the analysis of each stage in Region II, the results indicated that the green stage showed the greatest thermal stability for the two cultivars, while less thermal stability was observed in the ripe stage (Figure 1A,B). It should be noted that higher thermal stability was shown by the green stage of “Portola” (Figure 1C). The differences between the green and ripe stages with respect to thermal stability in Region II were recently explained as the effect of the different proteins and enzymes (such as xyloglucosyltransferase/endohydrolases, β-endoglucanases, and cellulases) with hydrolytic activity that cause polysaccharide solubilization and the degradation and depolymerization of the carbohydrate polymers of the cell walls of ripening fruit. In contrast, the polymers of the green stage are more ordered, assembled, and compact, probably due to the hydrogen bonds between cellulose and hemicellulose polymers providing a more highly ordered assembly and crosslinking the structural polysaccharides [24,26,31].

**Figure 1.** Thermogravimetric analysis (TGA) thermograms from different fruit stages of strawberry “Cristal” (A), “Portola” (B) or both (C) at temperatures between 50 and 550 °C.

The second analysis was differential thermogravimetric (DTG) analysis (Figure 2). Cell wall disassembly or cell wall component degradation has been described by an analytical Py-GC/MS study as a process that uses chemical fragmentation [32,33], or has been described as mediated by enzymatic processes [31]; these processes can be temperature-dependent [24,34–37]. The thermogravimetric curve of the DTG analysis is shown in Figure 2 and can be divided into four maximum degradation peaks, which occur between 125 °C or 170 °C and 375 °C. Previously, McGrath et al. (2003) [38] reported that at temperatures greater than 400 °C, thermogravimetry detected the presence of sample residue [24,25]. With respect to each curve area, at the first peak (approximately 125 °C or 170 °C), the green samples of “Cristal” showed a higher peak than the green sample of “Portola” (Figure 2A). The values of the first peak obtained between 125 °C or 170 °C in the 50% ripe samples showed that “Cristal” experienced a larger peak than “Portola” (Figure 2B); however, in the ripe samples, the curve showed a contrasting result to the other two stages, as the ripe “Portola” samples showed a larger peak than the ripe “Cristal” samples in the first peak (Figure 2C). This difference corresponds to the different carbohydrate polymers that, in natural conditions (without the effect of temperature), would be decomposing in each sample prior to reaching the degradation temperatures during the previously described TGA [24].
Figure 2. TG/DTG thermogram: (A) green stage of “Cristal” and “Portola”; (B) 50% ripe stage of “Cristal” and “Portola”; (C) ripe stage of both cultivars. The different graphs show the maximum degradation temperatures for the different stages of the two cultivars. In each graph, the red line shows “Portola” and the black line, “Cristal”.

The second peak, named the “A region”, showed that the samples of the green stage of “Portola” had a large, observable peak when compared to the “Cristal” sample, which had no peak (Figure 2A, black line). This result suggests that the “Portola” green-stage samples presented a higher depolymerization of cell wall polymers at this temperature (246.2 °C) than the “Cristal” green-stage samples (Figure 2A). A similar result was observed at the second peaks of the 50% ripe samples (Figure 2B); in contrast, for the ripe samples, smaller peaks were observed in the A region (Figure 2C).

An intermediate region was identified, and was named the “B region”, with a small peak at 274.4 °C and where the green samples and the ripe samples showed tendencies that contrasted with the A region. Notably, larger values were observed in the “Cristal” green-stage samples than in the “Portola” green-stage samples (Figure 2A), and in the “Cristal” ripe samples than in the “Portola” ripe samples (Figure 2C). In the “C region”, the values of the “Cristal” green samples were only slightly larger than those of the “Portola” green samples (Figure 2A). Additionally, the values of the “Cristal” ripe samples were larger than those of the “Portola” ripe samples (Figure 2C). In “Cristal” at the 50% ripe stage, the samples showed smaller values than “Portola” at the 50% ripe stage (Figure 2B).

Interestingly, Xiao et al. (2001) [39] showed that the decomposition of hemicelluloses fractions occurred between 200 and 300 °C; this region corresponds to Region II in the graphs of Figure 1, and to peak B in the graphs of Figure 2. Additionally, short-chain pectin fractions could be observed at approximately 250 °C [40], which corresponds to the “B region” in the graphs of Figure 2 and had a maximum value at 246.2 °C in the green stage (Figure 2A). This result was replicated in Figure 2B,C. The “C region” in the graphs of Figure 2 primarily shows the depolymerization of hemicelluloses [24,41,42]. Finally, the region over 350 °C (specifically between 360 and 400 °C) in Figure 2 principally shows the decomposition of lignin; to a lesser degree, cellulose can be seen at these temperatures, but this result was described as the secondary plant cell wall [24,41–43].

Regarding the percentage of cumulative depolymerization (PCD), the maximum degradation temperatures for the different stages of each cultivar were analyzed; these results were obtained from the first derivative of the TGA curve in Figure 2.

The correlation identified between the physicochemical ripening parameters, such as the firmness and soluble solid concentration (SSC), and the PCD value at different degradation temperatures is shown in Figure 3. The results showed a differential correlation between the two parameters in the different cultivars (Figure 3). “Cristal” did not show any correlation between the parameters, because while the SSC/firmness ratio increased, the PCD value did not show large changes in the green and 50% ripe stages (Figure 3A, black and red lines). Even in the ripe stage, the relationship seemed to be inversely proportional (Figure 3A). Regarding “Portola”, the relationship between the two parameters (PCD and SSC/firmness ratio) showed a good correlation, indicating that the PCD parameters demonstrated the process of cell wall depolymerization in the fruits as ripening progressed.
(Figure 3B). In this sense, we previously demonstrated that the SSC did not differ markedly during the ripening process of the two cultivars, while the firmness did [11]. For this reason, we propose that the effect of the changes in the thermal stability is more closely related to the firmness changes than the SSC. Additionally, differences between the different stages and the largest slope were observed in the analysis of the green samples with the equation defined as $y = 1.001x + 17.61$, followed by the 50% ripe stage with the equation defined as $y = 0.813x + 26.50$ (Table 1).

![Figure 3](image)

**Figure 3.** Correlation analysis between percentage of cumulative depolymerization (PCD) and fruit parameter (ratio Solid soluble concentration (SSC)/firmness) during three different stages of the “Cristal” (A) and the “Portola” (B). The SSC values were obtained from [11].

**Table 1.** Correlation equation obtained from Figure 3 analysis. N.D.: not determined.

|                   | Green Stage | 50% Ripe Stage | Ripe Stage |
|-------------------|-------------|----------------|------------|
| Cristal Equation  | $y = 0.2 + 25.3$ | $y = 0.1x + 31.9$ | $y = −0.1x + 47.2$ |
| $r^2$             | N.D.        | N.D.           | N.D.       |
| Portola Equation  | $y = 1.0x + 17.6$ | $y = 0.8 x + 26.5$ | $y = 0.2x + 47.1$ |
| $r^2$             | 0.994       | 0.973          | N.D.       |

Table 2 shows the PCD at each peak temperature per region defined in the graphs in Figure 2. Regarding “Cristal”, small or null changes were observed when passing from the 50% ripe stage to the ripe stage, and changes could only be seen between the green stage and the 50% ripe stage (Table 2). This result is possibly because the ripe fruit of “Cristal” has been described as a firmer fruit than “Portola” [24]; thus, deviations from the initial stage may not be very large. Regarding “Portola”, the PCD increased in each region or at each peak temperature as ripening progressed, and the fruit changed from one stage to the next. The PCD value at 335.1 °C, where 50.3% of the carbohydrate polymers present in the cell wall were depolymerized, indicated that only 46.1% had been polymerized during the green stage; thus, this stage had polymers that were more strongly assembled (Table 2). However, major differences can be seen at low temperatures: for example, at 246.2 °C, it is possible to see a peak in Figure 2A (described as “Region A”). At this temperature, 34.2% of the carbohydrate polymers present in the “Portola” cell wall were depolymerized, while only 18.6% had been polymerized at the green stage at the same temperature (Table 2).
Table 2. Percentage of cumulative depolymerization (PCD) for the three different regions calculated from the TG/DTG thermogram, compared with firmness and SSC values for “Cristal” and “Portola”. The SSC values were obtained from Ramos et al. (2018) [11]. Differences in firmness and SSC between mean ± standard errors (SE) (n = 20) were determined.

|                | 246.2 °C (Region A) | 274.4 °C (Region B) | 335.1 °C (Region C) | SSC (g/100 g FW⁻¹) | Firmness (N) | Ratio SSC/Firmness |
|----------------|---------------------|---------------------|---------------------|-------------------|--------------|------------------|
| Cristal Green stage | 24.3%               | 30.1%               | 46.5%               | 2.61 ± 0.27       | 3.61 ± 0.61  | 0.79             |
| Cristal 50% Ripe stage | 27.9%               | 35.2%               | 47.7%               | 5.43 ± 0.47       | 1.44 ± 0.22  | 3.78             |
| Cristal Ripe stage   | 27.1%               | 32.0%               | 45.8%               | 6.99 ± 0.44       | 0.72 ± 0.18  | 9.73             |
| Portola Green stage  | 18.6%               | 26.4%               | 46.1%               | 2.76 ± 0.26       | 4.00 ± 0.64  | 0.70             |
| Portola 50% Ripe stage | 22.8%               | 32.0%               | 50.1%               | 7.08 ± 0.46       | 1.24 ± 0.36  | 5.71             |
| Portola Ripe stage   | 34.2%               | 39.6%               | 50.3%               | 7.19 ± 0.56       | 0.44 ± 0.10  | 16.48            |

Interestingly, among the three temperatures obtained from the peaks in the A, B and C regions of the TG/DTG results in Figure 2, “Portola” showed the lowest values of firmness at the ripe stage, which was consistent with the increase in the SSC (described previously by [21]); furthermore, “Portola” showed high PCD values at 246 °C, 274 °C and 335 °C. Therefore, the “Portola” green stage showed the highest value for firmness [11]; however, the three PCD parameters of this stage were the lowest, indicating an inversely proportional relationship between the PCD parameters and firmness. However, regarding “Cristal”, this relationship was not as clear, since the differences in fruit firmness were not as marked [11] in regard to the degrees of wall degradation obtained by TGA. An important point is that cell wall polysaccharides are the principal component of the cell wall, but not the only component that constitutes and regulates cell wall structure: cell wall proteins also play an important role. Cell wall proteins may have the answer to the lack of correlation between depolymerization degree and thermal stability exhibited by the strawberry “Cristal”. However, to determine the putative effect of these cell wall proteins, future analysis is necessary.

3.2. SEM Studies of the Surface Morphology of “Portola” Strawberry

The changes in fruit firmness that take place during ripening are described as softening and changes in fruit texture; these changes can cause consumer rejection, and different studies have mainly related this phenomenon to alterations in the structure and/or composition of the cell wall [10–14]. To see this effect on the surface morphology of the two contrasting ripening stages of *F. x ananassa*, we used “Portola” as a representative case to show the two most contrasting stages through the use of SEM (Figure 4). The analysis revealed a complex ordered structure made of pectins, hemicellulose and cellulose (Figure 4A,B), and most importantly, it was possible to see the organized fibers as a whole (Figure 4A,B, white arrow). In contrast, the change in the cell wall surface morphology at the ripe stage was due to the disassembly or disorder of the polymeric matrix, associated with the ripening process, which promoted the decrease in firmness previously described in “Portola” and other cultivars [2,24]. Thus, a contrast in organization between the ripe and green stages was observed (see Figure 4C,D), showing the development of an amorphous or fragmented system.
Figure 4. Scanning electron micrographs (SEM) of *F. x ananassa* “Portola”. (A,B) are the green-stage samples; (C,D) are the ripe-stage samples. In (A,B), the white arrow shows the integrity of the cellulose microfibril. While in (C) the white arrow shows the disassembly of the cellulose–hemicellulose matrix; finally, in (D), the white arrow shows the putative cellulose microfibril. The samples were realized under different magnifications (A–C) 250×; (B–D) 500×.

4. Conclusions

The present work used thermogravimetric analysis (TGA) to examine the changes within the primary cell walls of two strawberry cultivars with contrasting softening ratios: the “Portola” strawberry, which is described as a very soft fruit, and the “Cristal” strawberry, which is described as a much firmer fruit. TGA showed the differences between the percentage of degradation of the two cultivars during the main developmental stages of the fruit, principally in Region II of Figure 1c, indicating that the green stage of “Portola” has the greatest thermal stability of the two cultivars. Notably, there was a positive relationship between depolymerization and the increased soluble solid concentration (SSC) and firmness ratio. The three PCD parameters were at their lowest at this stage, indicating an inversely proportional relationship between the PCD parameters and firmness of the two cultivars. Therefore, we conclude that depolymerization degree is inversely correlated with the thermal stability of the cell wall in the “Portola” strawberry fruit during its development; however, that correlation cannot be found for the “Cristal” strawberry.

**Supplementary Materials:** The following are available online at https://www.mdpi.com/article/10.3390/agronomy11061100/s1, Figure S1: Changes in fruit firmness during fruit ripening of the two contrasting strawberry (*Fragaria × ananassa*) cultivars.
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