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Characterizing the Impact of Postharvest Temperature Stress on Polyphenol Profiles of Red and White-Fruited Strawberry Cultivars

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Characterizing the Impact of Postharvest Temperature Stress on Polyphenol Profiles of Red and White-Fruited Strawberry Cultivars

by

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DEDICATION

I would like to dedicate this thesis to two individuals who mean a lot to me.

The first is my late grandfather Gerry Smith, who was so emphatic in telling me how proud he was of everything that I did with my life. I know you would have been the first person to congratulate me on completing my graduate degree and I miss you every day.

The second individual is my cat Kitty Baby who walked across my keyboard every single day while I wrote this and who encouraged me to finish this thesis and degree so that I can one day build her a full cat room.

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ABSTRACT

All living organisms have developed mechanisms that help them prevent internal stress and survive under harsh conditions. For fruits, specifically strawberries, stress is fought against primarily by increasing the synthesis of polyphenols, which are secondary plant metabolites with bioactive activity. Characterizing these bioactive compounds and the differences between strawberry cultivars can be vital for strawberry breeders. Furthermore, understanding the mechanisms that trigger the synthesis of polyphenols and their levels within different strawberry cultivars will provide breeders with tools to successfully identify cultivars with higher resistance to pre- and postharvest stressors. To gain that understanding, this study aimed to characterize the polyphenol profiles of three major Florida strawberry cultivars ('Florida Radiance, Sweet Sensation® 'Florida127', and 'Florida Brilliance') as well as a new white-fruited variety ('Florida Pearl') using an HPLC-DAD. First, to determine the impact of temperature stress on strawberries, 'Florida Radiance' and Sweet Sensation® 'Florida127' were stored at 1, 10, and 20°C and respiration rate (RR), water loss, total phenolics, anthocyanins, ascorbic acid, and total and individual sugar contents measured on the day of harvest and after 3 and 7 days of storage. Second, the impact of postharvest temperature stress on the polyphenol profiles of 'Florida Radiance' and Sweet Sensation® 'Florida127' was evaluated. Finally, polyphenol profiles were characterized, and total polyphenol and anthocyanin contents of 'Florida Brilliance' and 'Florida Pearl' were measured on the day of harvest and after 9 days at 1°C. To obtain an analytical color representation of the differences between the red and white-fruited berries, color attributes (L*a*b* system) were also measured for 'Florida Brilliance' and 'Florida Pearl'. Results showed that 'Florida Radiance' and Sweet Sensation® 'Florida127' cultivars stored at 20°C had higher respiration rates and water loss. They experienced a more significant decline in all secondary and primary metabolites than counterparts held at 1 or 10°C. Polyphenol profiles for 'Florida Brilliance' and 'Florida Pearl' were significantly different. Compared to 'Florida Brilliance', pelargonidin-3-glucoside was not detected in 'Florida Pearl', which seemed to have been "replaced" by a larger concentration of kaempferol-3-glucoside. Overall,

the results showed that strawberry cultivars differ significantly in their polyphenol profiles at harvest. Besides, postharvest temperature stress showed that higher stress temperatures lead to a greater decline in the polyphenol profiles and contents of primary and secondary metabolites. The effects of UV rays were indirectly studied in ‘Florida Brilliance’ and ‘Florida Pearl’ as preharvest stress. There was an evident change in the polyphenol profiles with an increasing UV index, particularly in the white-fruited strawberries. The results from this study can ultimately help with the further development of new strawberry genotypes. At the same time, they provide valuable information about the optimal growing and postharvest storage conditions for the white-fruited ‘Florida Pearl’.

CHAPTER 1: INTRODUCTION

With the continuous spread of citrus greening throughout Florida groves, the strawberry (*Fragaria* x *ananassa*) will soon rise to become the highest-grossing fruit within the state. Strawberries have been the focus of many studies for their health benefits due to high levels of bioactive compounds, including phenolic acids, known for their bioactive properties (Giampieri et al., 2014; Giampieri et al., 2012). The strawberry fruit contains many biochemical compounds, including primary metabolites such as sugars and ascorbic acid (vitamin C) and secondary metabolites such as polyphenols with antioxidant properties (Crecente-Campo et al., 2012; Nunes & Dea, 2016). Strawberry fruits contain three major sugars: sucrose, glucose, and fructose. Polyphenols are a large family of secondary metabolites synthesized via the phenylpropanoid pathway, a highly branched pathway, and constitute the plant's defense system. The primary polyphenol class in strawberry fruit are anthocyanins, which belong to the large flavonoid group. The flavonoid pathway begins with chalcone synthase (CHS), catalyzing 4-courmaroyl CoA and 3-malonyl CoA into chalcone (Fig. 3). Anthocyanins give the strawberry its signature red color and are synthesized to protect the fruit from UV radiation. The color of anthocyanins is pH-dependent, with the more alkaline fruits having a deep purple color, such as blueberries. Further, the levels of polyphenols in different strawberry genotypes can be ultimately used as a selection indicator for genotypes with increased stress resistance and health benefits.

Strawberries are highly perishable, so quality is best maintained if the fruit is stored at its optimum temperature (0-1°C). When the fruit is stored under stress-inducing temperatures, it has been noted that the primary metabolites such as sugars will break down significantly faster and at a higher rate (Nunes & Dea, 2016). However, regarding the change in polyphenol profiles, studies show variable results. As strawberries are kept in storage (either at optimum or abuse temperatures), their color changes to a darker shade of red. As anthocyanins are responsible for the red color of the fruit, one could expect that a deeper red color would result from increased anthocyanin levels during storage. Thus, suggesting that the synthesis of these compounds would continue after the fruit has been detached from the mother plant

and during storage. However, there is a lack of information on the impact of postharvest temperature stress on the polyphenol profiles of different strawberry cultivars. Furthermore, few studies on white-fruited strawberries have shown their possible correlation with reduction in the hypoallergenic reactions otherwise caused by red-fruited strawberries. However, no studies have characterized the polyphenol profiles and the postharvest performance of white-fruited strawberries, particularly ‘Florida Pearl’.

Therefore, the objectives of the work presented in this thesis were 1) to investigate the biochemical changes on ‘Florida Radiance’ and ‘Sweet Sensation’® ‘Florida127’ strawberry cultivars during postharvest storage at both stress and optimal temperatures, 2) to determine the differences in polyphenol profiles between ‘Florida Radiance’ and Sweet Sensation’® ‘Florida127’. Further, changes in the polyphenol profile within each cultivar due to postharvest stress (i.e., time-temperature stress) were evaluated, and 3) to characterize and compare the polyphenolic profiles of the commercial standard, red-fruited strawberry ‘Florida Brilliance’ and first Florida white-fruited ‘Florida Pearl’ strawberry at harvest and during postharvest. This work will ultimately provide insight into possible mechanisms of postharvest stress resistance in strawberries and the significant differences in polyphenol profiles between cultivars.

CHAPTER 2: REVIEW OF LITERATURE

Agricultural and Commercial History of the Strawberry

Strawberries are grown commercially in 76 countries worldwide, with the United States being among the top five producing nations globally (Simpson, 2018). Berries as a whole have been grown as a food source for over 2000 years, and even before the development of agriculture, they would have been an essential part of human diets (Simpson, 2018). The first hybrids of the strawberry *Fragaria* × *ananassa* were produced during the 18th century. Still, the first cultivar known as 'Keen's Seedling' (Fig. 1) and considered as the "modern strawberry" was bred and released in 1821 (Simpson, 2018). Diving deeper into the breeding of the modern strawberry, the first documented cross which led to what is believed to be *F. ananassa* was in France with the crossbreeding of *F. chiloensis* and *F. virginiana*. As their names suggest, *F. chiloensis* was a variety from Chile, and *F. virginiana* was from the Americas (Darrow, 1966). A variety of *F. virginiana*, the 'Scarlett Strawberry', was popular in England, and with the successful strawberry breeding in France, many English gardeners tried to "better" the Scarlett (Darrow, 1966). Two Englishmen are now most widely recognized as bringing about the modern strawberry breeding practice: Thomas Andrew Knight and Michael Keens. Knight developed a more scientific approach to breeding new varieties as he worked with the Royal Horticultural Society (RHS). Keens also worked with the RHS; however, he did not begin doing so until submitting his breed, the 'Keens Imperial', to the RHS (Darrow, 1966). Since the development of this parent hybrid, hundreds of strawberry cultivars have been bred and commercialized worldwide.

In the United States, the top two states in strawberry production are California and Florida (Samtani et al., 2019). Florida, specifically Plant City, is considered the winter strawberry capital of the world. The strawberry harvest season starts in early December through May, with most fruits harvested and sold between February and March. The strawberry is Florida's second-highest production value crop behind citrus (Samtani et al., 2019). The continued issue of citrus greening could encroach strawberries even more upon citrus. With profitability increasing in the strawberry industry, breeding cultivars that best fit the current

Florida climate and potential future climate changes have become even more critical. Currently, ‘Florida Brilliance’ is the highest yield short-day strawberry cultivar in Florida (Whitaker & Fan, 2020), but several other cultivars are being tested and commercialized every year.

Figure 1: The ‘Keens Seedling’. The ‘Keens Seedling’ was bred by Michael Keens in England and released in 1821. This strawberry is regarded as the predecessor to the modern strawberry, and most modern strawberries can trace their parentage back to the ‘Keens Seedling’ (Simpson, 2018).

Strawberry Varieties

The commercial strawberry we know today is *Fragraria* × *ananassa* and is an octoploid fruit. Within *Fragaria* × *ananassa,* there are many different breeds, or cultivars, from different origins worldwide. In Florida, several cultivars are grown during the season, with the most prevalent being ‘Florida Radiance’, otherwise known as Radiance. Sweet Sensation®‘Florida127’ (Sensation) is the second most pervasive

strawberry cultivar, with 'Florida Brilliance' (Brilliance) currently becoming the dominant cultivar grown in Florida (Samtani et al., 2019). All three strawberry cultivars vary in physical appearance and their biochemical makeup. The newest Florida-bred strawberry cultivar and the most distinct is 'Florida Pearl', a white strawberry variant. All four varieties are bred and cultivated by the University of Florida at the Gulf Coast Research and Education Center in Wimauma, Florida.

'Florida Radiance'

As of 2019, Radiance was the dominant cultivar grown in Florida, with Sensation being a close second among Florida growers (Samtani et al., 2019). Radiance was commercially released in 2009 and became a complementary cultivar to the 'Strawberry Festival' (Chandler et al., 2009). The direct parents of Florida Radiance were 'Winter Dawn' and FL 99-35, which were both bred previously by the University of Florida (Chandler et al., 2009). Radiance has a bright red coloring that darkens over time but stays a very vibrant red and has both larger-sized fruit and a high yield per plant (Chandler et al., 2009). Overall, Radiance was deemed similar enough to Festival to become a major cultivar in Florida.

Sweet Sensation® 'Florida127'

Strawberry Sensation was trialed as a selection in 2009 while breeders were looking for another strawberry cultivar with a high yield as Radiance (Whitaker et al., 2015). The direct parentage of Sensation consists of Winterstar™'FL 05-107' (the female parent) and FL 02-58 (the male parent), which were both bred previously at the University of Florida (Whitaker et al., 2015). Sensation was commercially released in 2015 after three consecutive years of field performance testing and laboratory biochemical testing (Whitaker et al., 2015). A trained sensory panel was also utilized during that time to determine the differences between Sensation, Festival, and Radiance in terms of appearance and taste (Whitaker et al., 2015). Sensation was shown to have a high fruit yield, but the fruit itself was also large, similar to Radiance. As stated previously, as of 2019, Sensation and Radiance were the dominant commercial cultivars replacing Festival (Samtani et al., 2019).

'Florida Brilliance'

'Florida Brilliance' originated from a cross between two unreleased strawberry cultivars from the University of Florida: FL 11.31-14 (female parent) and FL 10-153 (male parent) (Whitaker et al., 2019). Brilliance was bred and first planted in 2013 and was given its commercial cultivar name in 2017 (Whitaker

et al., 2019). Before its commercial release in 2019, the variety was studied for three consecutive harvest seasons (2015-2018) for its field performance and biochemical content during postharvest (Whitaker et al., 2019). Brilliance is a firmer fruit than Radiance or Sensation, having a better performance during postharvest handling and storage (Whitaker et al., 2019). In 2020, the cultivated acreage of Brilliance increased significantly, and it became the leading strawberry cultivar in Florida.

'Florida Pearl'

'Florida Pearl' is the most recent Florida cultivar, and it stands out from other strawberry cultivars because the fruit stays white-pinkish when fully ripe. The white strawberry is a naturally occurring variety originally named *Fragaria chiloensis*. The fruit originated in Chile as a wild forest growing berry and has been cultivated and bred to create different strawberry hybrids suitable for growing commercially in other environments (Morales-Quintana & Ramos, 2019). The white-fruited strawberry is one of the parental varieties crossed to create *Fragaria X ananassa* (Morales-Quintana & Ramos, 2019). *Fragaria chiloensis* has a lower content of the Fra a 1 protein, thought to be an allergen protein found in higher levels in red-fruited strawberries than in the white-fruited varieties (Franz-Oberdorf et al., 2017). Thus, the white-fruited strawberries are thought to have a lower potential to cause allergic reactions. However, as the Fra a 1 gene is not entirely naturally knocked out, there is still a small production of those allergens (Franz-Oberdorf et al., 2017). The lower Fra a 1 is also linked to the berries' white/pink coloring. Munoz et al. (2010) determined that the Fra allergen does have some function within the flavonoid biosynthesis pathway, leading to a decrease in the anthocyanin synthesis when its production is downregulated (Munoz et al., 2010). With white strawberries having the potential to be hypoallergenic and becoming very trendy in gourmet markets and on social networking, further research into best agricultural practices and postharvest handling conditions could bring a significant gain for the strawberry industry.

Important Strawberry Chemical Components

The strawberry fruit contains many biochemical compounds, including primary metabolites such as sugars and ascorbic acid (vitamin C) and secondary metabolites such as polyphenols with antioxidant properties (Fig. 2). The amount and type of the different polyphenols and the levels of other chemical

components such as sugars and ascorbic acid vary between strawberry cultivars and, are greatly influenced by environmental conditions during growth.

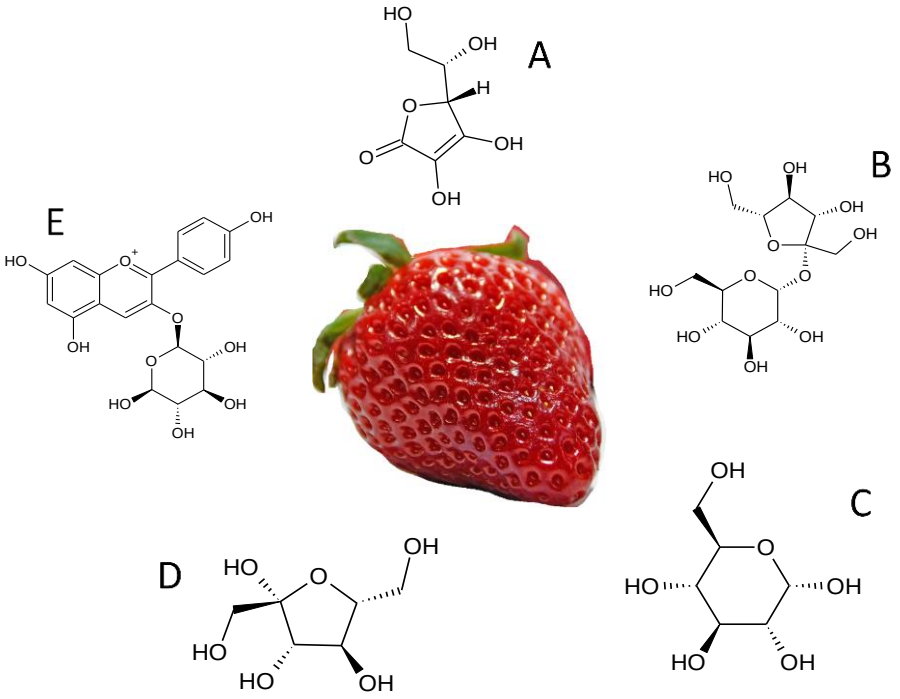

Figure 2: The Major Biochemicals Present in Strawberries. The figure represents a single strawberry surrounded by the chemical structures of the major primary and secondary metabolites present within the fruit: Ascorbic acid (A), Sucrose (B), Glucose (C), Fructose (D), and Pelargonidin-3-glucoside (E). A and E are considered secondary metabolites, while B, C, and D are primary metabolites.

Sugars

Strawberry fruit contains three major sugars: sucrose, glucose, and fructose (Cordenunsi et al., 2002). While the amounts of each sugar differ between the strawberry varieties, all three sugars usually are present within the fruit at harvest (Cordenunsi et al., 2002). Sugars constitute a significant metabolite within the fruit but degrade quickly after being harvested and during postharvest storage. Sugars are a part of the pentose-phosphate pathway as glucose-6-phosphate is needed to start the pathway being acted upon by glucose-6-phosphate dehydrogenase (Alfarouk et al., 2020). The primary function of this metabolic pathway is to provide the plant cells with NADPH and pentose phosphates to help maintain the cell's redox state as

the two phases of the pathway are oxidative and non-oxidative. Plants are also producers, meaning they can synthesize all the primary metabolites they need to sustain their metabolism. The high sugar content is suitable for the fruit's metabolic pathways, provides energy for metabolic reactions, and is also an important contributor to the quality and sensory characteristics of strawberry fruits. Usually, sweeter fruits have a greater acceptance from consumers.

Ascorbic Acid

Ascorbic acid, otherwise known as vitamin C, is an essential biochemical compound in strawberry fruit. The main synthetic pathway is the Smirnoff-Wheeler (SW) pathway, but several variant pathways have been proposed. In strawberries, ascorbate is synthesized with a combination of the SW pathway (L-galactose pathway) and a D-galacturonate pathway. Each ripening stage of the fruit seems to regulate the synthesis of ascorbic acid, determining which of the pathways is activated (Aguis et al., 2002). It has been shown in previous studies that the amount of ascorbic acid can differ between strawberry cultivars (Cruz-Rus et al., 2011). Like the sugars, ascorbic acid degradation is very fast after the fruit is harvested and during postharvest handling and storage. Ascorbic acid is the biochemical that decays at the most rapid rate within the strawberry fruit, beginning to oxidize almost immediately after detaching the fruit from the plant. Ascorbic acid is generally known for its importance in the human diet. However, ascorbic acid is also very beneficial and vital for the well-being of plants and fruits. It helps combat reactive oxygen species, which cause oxidative stress within the fruits. Ascorbic acid concentration can also be dependent upon the other metabolites and chemicals present within the tissues of the fruit. Thus, the concentration of ascorbic acid can be a good marker for the overall nutritional value of strawberry fruit. Additionally, as ascorbic acid oxidizes rather quickly postharvest, this compound constitutes a good biomarker and is often used to determine the postharvest quality of fruits and vegetables.

Polyphenols

Polyphenols are a large family of secondary metabolites synthesized via the phenylpropanoid pathway, a highly branched pathway that constitutes the plant's defense system (Fig. 3). Once considered waste generated by primary metabolism, it is now accepted that polyphenols have a critical role in plants. They serve as cell wall constituents, pollinator attractants, seed dispersal attractants, signal transducers, antioxidants, and a defense system against many biotic and abiotic stresses (Sarkar and Shetty, 2014). In

terms of structure, while there is a large variety of these biomolecules, all derive from the deamination of either phenylalanine or tyrosine, which delivers the basic phenylpropanoid unit C_6-C_3. They are classified based on their wide range of chemical structures and biological functions and are believed to accumulate in the vacuole of plants, usually as glycosides or other conjugates (Strack and Sharma, 1985).

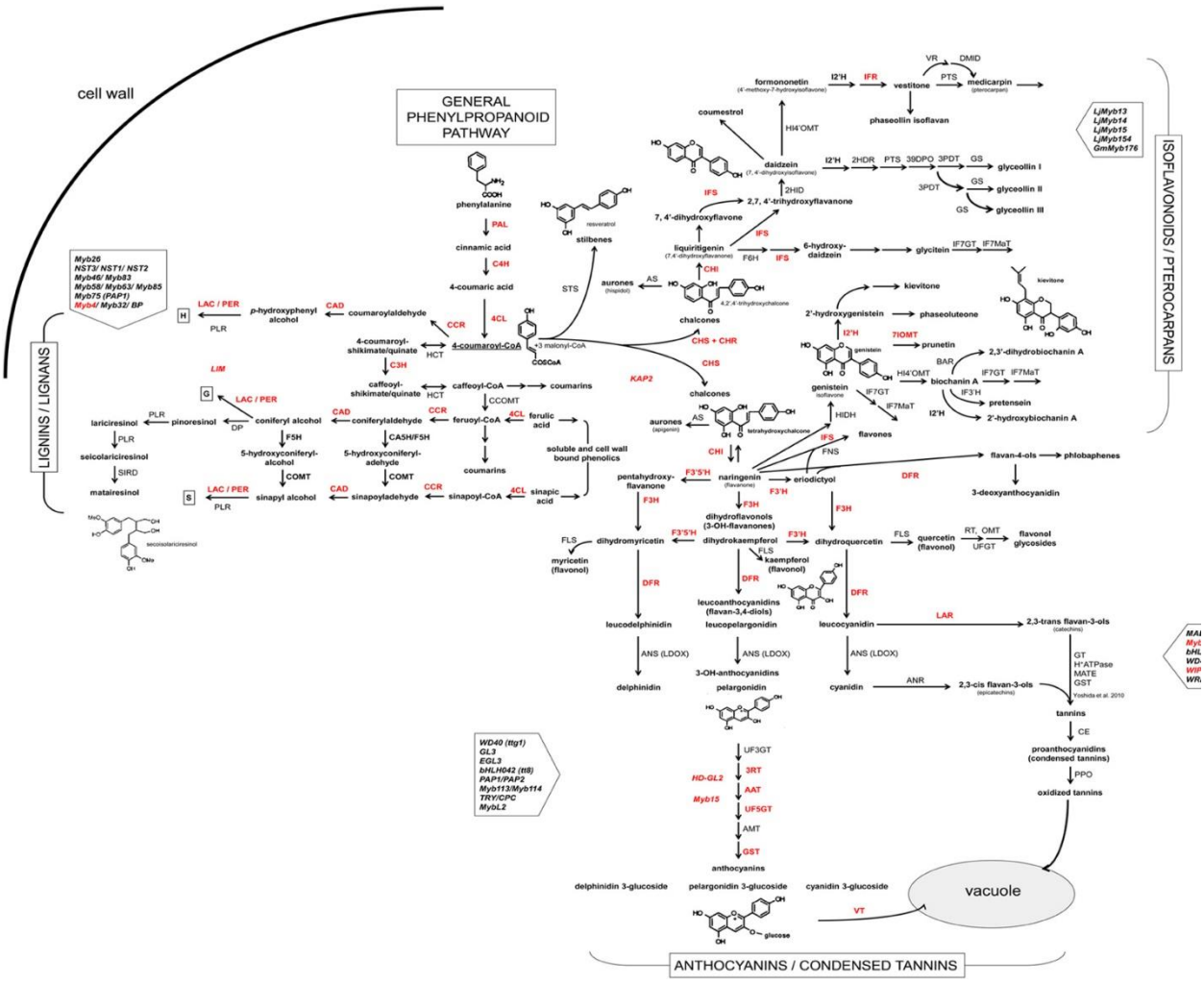

Figure 3: Phenylpropanoid Pathway [(Heller and Forkmann (1988); Reinprecht et al. (2016)]. The figure depicts a flowchart diagram of the phenylpropanoid pathway, including all regulatory enzymes and polyphenols. The pathway begins with phenylalanine-ammonia lyase (PAL), catalyzing the conversion of L-phenylalanine to trans-cinnamic acid.

Strawberry polyphenols have been extensively studied due to their acclaimed bioactive properties. Flavonoids are the primary polyphenols in strawberries and are commonly found in the tissues conjugated

to glucose or rhamnose (Clifford, 2000; Laura et al., 2009). For example, anthocyanidins are the non-glycosylated or aglycone forms of the anthocyanins, with pelargonidin-3-glucoside and cyaniding-3-glucoside being the most abundant anthocyanins found in strawberries (da Silva Pinto et al., 2008; Lopes-da-Silva et al., 2002) (Fig. 4). Anthocyanins give the strawberry its signature red color and are mainly synthesized to protect the fruit from UV radiation. The color of anthocyanins is pH-dependent, with the less acidic fruit (e.g., blueberries) having a deep purple color. The only flavonoids in strawberries in the non-glycosylated monomer form are the flavan-3-ols catechin and epicatechin. The flavonols quercetin and kaempferol and their glycosylated counterparts have also been identified in different strawberry varieties (da Silva et al., 2007; Vallejo et al., 2004) (Fig. 4).

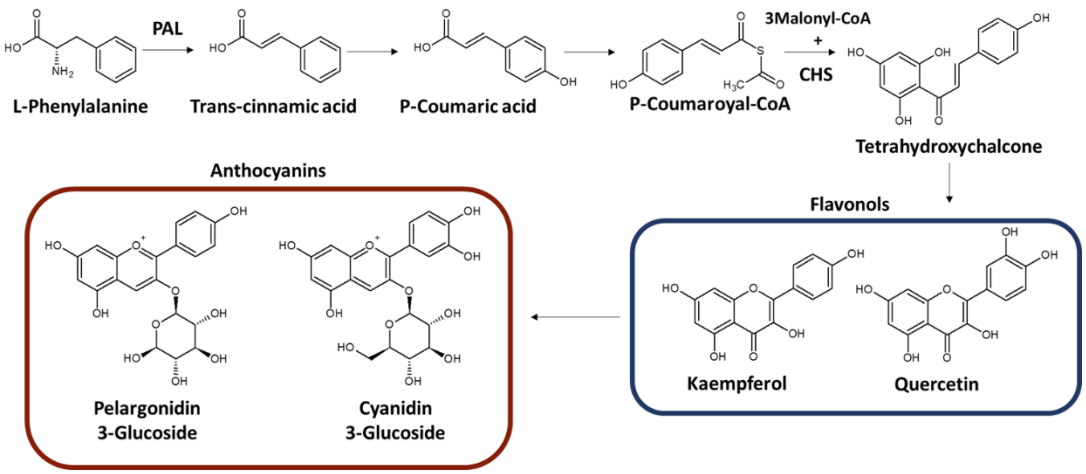

Figure 4: Condensed Phenylpropanoid Pathway. The flow-chart type diagram shows a simplified phenylpropanoid pathway. The two enzymes shown are phenylalanine ammonia-lyase (PAL) and chalcone synthase (CHS). PAL catalyzes the change of L-phenylalanine to trans-cinnamic acid and is a key regulatory enzyme at the beginning of the pathway. CHS catalyzes the reaction, which forms tetrahydroxychalcone and starts the branch of the primary polyphenols in strawberries, the flavonoids. The flavonols and anthocyanins are a part of the flavonoid branch with the major chemicals of each classification, within strawberries, shown.

Strawberries also contain flavanones, isoflavones, and non-flavonoid compounds such as phenolic acids (Laura et al., 2009). Phenolic acids present in significant amounts in strawberries include *p*-coumaric, ferulic, and caffeic acids (Aaby et al., 2007). Also, hydrolyzable tannins, mixtures of polygalloyl glucose, and poly-galloyl quinic acid derivatives containing gallic acid residues have been isolated from strawberries (Ishikura et al., 1984). The most common hydrolyzable tannins are found in plant tissues as simple esters

of glucose, tartaric acid, and quinic acid (Laura et al., 2009; Rice-Evans et al., 1996). Plants may produce ellagic acid from hydrolysis of tannins such as ellagitannin and gallic acid (Laura et al., 2009). Ellagic acid has also been detected in strawberries in significant amounts (Aaby et al., 2012; Häkkinen et al., 2000). Other non-flavonoid compounds found in minute quantities in strawberries include coumarins, benzophenones, xanthones, stilbenes, chalcones, lignans, secoridoids, and acetophenones (Aaby et al., 2007).

The major regulatory enzymes in the phenylpropanoid pathway include phenylalanine ammonia-lyase (PAL), which starts the pathway, chalcone synthase (CHS), which starts the flavonoid branch of the pathway, and polyphenol oxidase (PPO), which degrades the polyphenols at the end of the pathway. PAL activity is induced when a plant is exposed to extreme biotic and abiotic changes in the environment, arising from variations in temperature, drought, light, nutrient deficiency, or pathogen attack (Dixon and Paiva, 1995). For example, light has an important role in the color development of strawberries. When they are grown under a UV blocking film compared to open-field, the content of cyanidin 3-glucoside, quercetin 3-glucoside, and kaempferol 3-glucoside is lower (Josuttis et al., 2010). Studies show that when fruits are detached from the plant, conditions after harvest also significantly impact PAL and CHS activities. For example, Jin et al. (Jin et al., 2016) reported a significant increase in PAL activity of strawberries exposed to 45°C when compared to non-heated fruit. In contrast, others showed that exposure of strawberries to 42 or 48°C for 3 h results in a reduction of anthocyanin accumulation and PAL activity and a decrease of protein synthesis, compared to fruit kept at 0°C (Civello et al., 1997). Induction of PAL and CHS activities and the resulting accumulation of anthocyanins were also observed when strawberries were exposed to blue light irradiation (Xu et al., 2014) or cultivated under red and yellow light-quality selective plastic films (Miao et al., 2016). While PAL is involved in the synthesis of polyphenols, the enzyme polyphenol oxidase (PPO) is responsible for their oxidation and degradation. PPO performs two reactions: the o-hydroxylation of monophenols and the oxidation of diphenols to o-quinones (Nunes et al., 2005).

When exposed to biotic or abiotic stress, plants can synthesize thousands of secondary metabolites via the phenylpropanoid pathway. Production of such secondary metabolites constitutes the plant defense mechanisms (Dixon & Paiva, 1995). Polyphenols play three prominent roles within the fruit: cell wall constituents, pollinator attractants, and stress defense. For cell wall constituents, lignin is the primary

polyphenol responsible for providing structural integrity to the cell wall aiding cellulose (Boerjan et al., 2003). For strawberries specifically, lignin is not present in significant amounts within the fruit's flesh; therefore, the fruit mainly relies on pectin to maintain its rigidity. The next role of polyphenols in fruits is pollinator attractants (and seed dispersal). Brighter colors are more likely to attract animals to the plants, so the primary polyphenols responsible for this role are the flavonoids and, in strawberries, specifically the anthocyanins like pelargonidin-3-glucoside (Bartley et al., 1982). Anthocyanins also play a role in UV protection for the fruit, darkening the color to help prevent excess production of reactive oxygen species (Smillie & Hetherington, 1999).

Abiotic Stress for Strawberries

Consumption of berries has been correlated with decreased risk of many degenerative diseases, attributable to components such as fiber, vitamins, and polyphenols. Berries are also excellent sources of bioactive compounds such as vitamin C and polyphenols, namely flavonoids (anthocyanins, flavonols, and flavanols), phenolic acids, and tannins. Amongst berries, strawberries can contribute substantial amounts of bioactive compounds to the diet. Thus, their impact on human health can be significant (Giampieri et al., 2012; Hannum, 2004; Szajdek and Borowska, 2008; Zafra-Stone et al., 2007). However, overall strawberry quality and the levels of bioactive compounds are greatly influenced by agricultural practices (e.g., conventional or organic cultivation) and postharvest conditions (e.g., storage time, temperature, and humidity) that can cause stress on the plant and impact the quality of the fruit.

In biological terms, stress can be defined as a force that inhibits an organism from functioning normally (Mahajan & Tuteja, 2005). The major abiotic stressors for strawberries can be classified into pre-harvest and postharvest stressors. Important pre-harvest stressors are UV light, rainfall (excess water), drought, soil composition, and environmental temperature. Postharvest stressors include storage temperature and relative humidity (RH), and time-temperature stress. Improper postharvest storage conditions can lead to the production of reactive oxygen species, which causes oxidative stress to the fruit.

Pre-harvest Abiotic Stress and the Impact on Strawberry Quality

Environmental Temperature and UV Stress

Plants have preferred optimal growing temperatures. The hardiness scale, developed by the United States Department of Agriculture (USDA), is utilized to help growers identify the best areas within the United States to grow certain crops. For example, strawberries can be grown as perennial fruits in zones 5-8 (-28.9 to -6.7°C) or as winter season crops in zones 9 and 10 (-6.7 to 4.4°C). Temperatures outside of the optimum range can disrupt the normal growth of the plant. Too high temperatures can reduce strawberry plant and fruit growth and cause abnormal leaf color (Wang & Camp, 2000). Too low temperatures can also cause irregular plant and fruit growth, affecting fruit quality. Freezing or ice on the plant/fruit can cause cell membrane breakdown and disturb overall growth (Y. Zhang et al., 2019).

As stated previously, all plants need light to grow, but the quality of the light impacts the physical and nutritional quality of the fruits produced. While plants need light exposure to grow and produce energy, too intense light exposure can damage the fruit due to UV rays. Plants must find ways to protect themselves from UV radiation as the rays can cause cellular damage, leading to lower quality fruits at harvest (Giampieri et al., 2012; Smillie & Hetherington, 1999; Steyn et al., 2002). For example, there can be a difference in flavonoid production for fruits exposed to different wavelengths of visible light (Zoratti et al., 2014b). It has also been shown that there is an increase in chalcone synthase activity (a major enzyme within the phenylpropanoid pathway that starts flavonoid synthesis) when plants are exposed to higher UV rays (Cominelli et al., 2008). This increase in chalcone synthase activity is most likely a result of the plant trying to protect itself from harmful UV rays, which increases the overall content of the fruit UV natural protectant, the anthocyanins (red pigments).

Water Stress

Regarding water stress, two different extreme conditions can occur during the growing season: flooding and drought. Flooding (i.e., excess water) results in reduced oxygen supply to the roots of the plants, leading to a limitation of nutrient intake (Mahajan & Tuteja, 2005). Drought (i.e., water deficit) is the most common water stress for plants. It can lead to a breakdown of plant cells' outer membrane, ending in potential membrane protein/enzyme disruption (Mahajan & Tuteja, 2005).

While total flooding is rare during the strawberry season in Florida, regular rainfall can impact the fruit quality. Excess rain can cause severe damage to the delicate skin of the strawberry fruit, such as cellular breakdown and increased respiration rate (Herrington et al., 2011). There is a difference between strawberry cultivars in how rainfall impacts fruit physicochemical characteristics. It is primarily due to a difference in the turgidity of the outer flesh of the fruit (Herrington et al., 2011). As stated briefly, one of the most significant impacts of excess rainfall can be the increased respiration rate of the fruit. This increased respiration rate results from bruising on the berries (Chang & Brecht, 2020), a significant physical effect of rainfall on the fruit (Herrington et al., 2011). The combination of increased RR and cellular breakdown can lead to substantial depletion of nutrients, resulting in the decreased nutritional value of the fruit due to increased metabolism and a decrease in postharvest shelf life.

Soil Composition

Salt stress, phosphate starvation, and soil pH are primary stress conditions affecting plant growth. Soil salinization, or the accumulation of water-soluble salts within the soil, is a global issue that affects ~24% of all irrigated land worldwide (Thomas & Middleton, 1993). This accumulation occurs when evaporation exceeds precipitation (Thomas & Middleton, 1993), and climate changes aggravate this condition. Phosphate starvation is the lack of inorganic phosphate (Pi) available within the soil of the irrigated land (Rouached et al., 2010). While plants evolve and develop mechanisms to overcome this stressor, the lack of available Pi can still cause abnormal plant growth (Rouached et al., 2010). Finally, the pH of the soil can significantly affect plant growth. For strawberry plants, the optimum soil pH should be slightly acidic, ranging from 4.6 to 6.5 (Milosevic et al., 2009). However, below that pH 4.6 threshold, the yield of the strawberry fruit is significantly lesser than that at the higher end of the pH range (Milosevic et al., 2009). Too alkaline soil would most likely have the same outcome as the environment would not be the best for the strawberry roots to uptake nutrients.

Postharvest Abiotic Stress and the Impact on Major Strawberry Chemical Compounds

Strawberries are highly perishable, so quality is best maintained if the fruit is held between 0 and 1°C with 90% humidity. Strawberries are often exposed to abiotic stresses, abusive temperatures, and low humidity during the supply chain, with large quantities of strawberries usually discarded. Those that

ultimately are consumed fresh may have lost up to 50% of their potential “bioactive quality” due to poor temperature management. Therefore, even though the initial bioactive quality of berries may be high at harvest, a significant amount of the health-promoting compounds is often lost before consumption. Stressful conditions accelerate the metabolism, lead to excessive moisture loss, and significantly decline sensory quality, sugars, vitamin C, and polyphenols**.**

Storage Temperature

All fresh fruits and vegetables have optimum postharvest storage conditions, including the best temperature to extend their shelf life (Horvitz, 2017). For strawberries, that temperature is from 0 to 1°C, but most experience 4 to 20°C on their journey from field to the table (Horvitz, 2017; Kelly et al., 2019). Improper postharvest handling temperatures affect the strawberry fruit's overall appearance due to water loss (i.e., color and texture) and the levels of sugars and bioactive compounds (i.e., ascorbic acid and polyphenols). Improper storage temperatures can severely affect the quality of the fruit and its shelf life, decreasing it drastically (Horvitz, 2017; Kelly et al., 2019).

As a primary metabolite, sugars are hydrolyzed significantly faster when the strawberry fruit is stored at stress-inducing temperatures (i.e., temperatures above the optimum). Along with water content, sugars are greatly affected by the higher respiration rates of strawberries. The increase in the overall rate of metabolic reactions (e.g., glycolysis) with increased storage temperature might also contribute to the breakdown of sugars, depleting fruit reserves.

Ascorbic acid is a major biochemical within the strawberry fruit, and it serves an essential role in combating reactive oxygen species (ROS). The more stress the strawberry fruit undergoes, the greater the ascorbic acid degradation. Both pre-harvest and postharvest conditions can affect the degradation of ascorbic acid. The most significant pre-harvest factor is exposure to UV radiation. Excess UV rays can lead to an increase in reactive oxygen species due to cellular breakdown. During postharvest, higher temperatures may lead to a faster ascorbic acid breakdown and increased ROS due to cellular breakdown.

Storage temperature also has a significant impact on strawberry polyphenols. For example, in one study, strawberries stored at abuse temperatures (>0°C) had higher levels of polyphenols, namely anthocyanins, during storage (Ayala-Zavala et al., 2004). Other studies suggested that the increase in certain polyphenols could have resulted from synthesis during postharvest, whereas others attributed the

increase in polyphenol contents to the degradation of fruit cell structures and consequently to a higher extractability of the compounds (Azzini et al., 2010; Piljac-Žegarac and Šamec, 2011). Furthermore, some reported that low storage temperature did not affect the levels of flavonols, ellagic acid, and total phenolics but contributed to a delay in synthesizing anthocyanins (Cordenunsi et al., 2003; Cordenunsi et al., 2005).

Since the data from most of these studies are reported in fresh weight, a concentration effect may also cause the increase reported in certain polyphenol compounds due to loss of water during storage, rather than to an actual increase. Thus, to determine the real changes in bioactive compounds and to show the differences between temperatures that might be obscured by differences in water content, concentrations of chemical constituents should be expressed in dry weight. Shin et al. (Shin et al., 2008) reported that the marked decline in total flavonoid and phenolic concentrations in ripe red fruit after 12 days of storage at 10°C paralleled the increase in water loss and decreased anthocyanin concentrations. Goulas and Manganaris (2011) also reported the bioactive composition of strawberries in dry weight. They showed a progressive decrease in hydroxycinnamic acid derivatives during storage at 20°C but inconsistent changes in other bioactive compounds.

Relative Humidity (RH)

The relative humidity (RH) relates to the moisture in the environment during postharvest handling of strawberry fruit from the field to the consumer's home. Relative humidity plays a significant role in the shelf life of fresh fruits and vegetables as it can either increase or decrease the transpiration of the fruits. For strawberries specifically, a lower RH significantly increases the rate of transpiration in the fruits, which in turn increases the loss of moisture and can decrease their shelf life (Shin et al., 2007; Shin et al., 2006; Shin et al., 2008; Sousa-Gallagher et al., 2013). The length of time in postharvest storage is not necessarily an abiotic stressor on its own but is tied in with both RH and temperature. The longer the strawberries stay under stressful storage environments, the faster they deteriorate. A higher transpiration rate can lead to more significant weight loss during postharvest storage and, greater weight loss can lead to significant decreases in the nutritional quality of the fruits (Nunes & Emond, 2007; Sousa-Gallagher et al., 2013). RH is also generally tied to storage temperature, with a higher RH having the potential to allow for slightly increased shelf life for berries stored at sub-optimal temperatures (Sousa-Gallagher et al., 2013).

CHAPTER THREE:

BIOCHEMICAL APPROACH TO DETERMINE THE IMPACT OF POSTHARVEST ABIOTIC STRESS ON STRAWBERRIES

Introduction

Strawberries are the most consumed berry fruit worldwide due to their attractive appearance, flavor, and nutritional properties. Plant City, in Florida, is recognized as the Winter Strawberry Capital of the world, producing most of the strawberries grown in the US between November and early April (Giampieri et al., 2014). Delivering strawberries at the highest quality from Florida to distant markets is difficult due to the delicate nature of the fruit. The optimum postharvest handling and storage conditions for strawberries are well established at temperatures ranging from 0°C to 1°C and 95% relative humidity (RH). However, these conditions are challenging to maintain throughout the supply chain (Pelletier et al., 2011). Besides, even with optimal conditions met, the strawberry has a maximum shelf life of fewer than two weeks (Knee, 2002).

The effect of postharvest temperature stress has been well documented for many different fruits and particularly for strawberries (Ayala-Zavala et al., 2004; Pelletier et al., 2011; Rapisarda et al., 2001; Shin et al., 2007; Shin et al., 2006). When stored outside of their optimal temperature and RH range, the overall physical and biochemical quality of strawberries decline significantly due to increased enzymatic activity, depletion of fruit chemical reserves which ultimately leads to senescence, poor overall quality, and reduced shelf-life. For example, Shin et al. (2007) showed that strawberries exposed at 20°C had a significant decrease in ascorbic acid and overall quality compared to fruit stored at optimum temperature. The same authors also showed that relative humidity (RH) too had a significant impact on the quality and biochemical content of the fruits during postharvest storage. The higher the storage RH, the minor weight loss, and the lesser the decrease in total phenolic and ascorbic acid contents. Another study conducted by Muley and Singhal (Muley & Singhal, 2020) to determine the effect of a chitosan-whey protein coating on the quality of strawberries stored at different temperatures showed a significant difference in ascorbic acid, total phenolics, and reducing sugars (fructose and glucose) contents between samples stored at 5°C and 20°C.

Overall, the fruit stored at the higher temperature had a more significant change in the quality, and the strawberry samples held at 20°C showed more significant weight loss than the samples stored at 5°C.

Although it is well established that storage temperature and relative humidity (RH) significantly affect the overall quality of strawberries, the response to temperature stress between different Florida strawberry cultivars is not well documented. Kelly (2018) showed a significant difference in specific biochemical attributes between Florida strawberry cultivars (i.e., Radiance, Sensation, and Beauty), but the study was conducted using only an optimum storage temperature (≈ 1°C). Still, no data shows the effect of stress temperatures on the biochemical characteristics of 'Florida Radiance' and Sweet Sensation'® 'Florida127' strawberries, two genetically different cultivars and with almost opposite biochemical profiles. 'Florida Radiance' has on average lower sugar contents and higher total phenolics, anthocyanins, and ascorbic acid than Sweet Sensation'® 'Florida127', which has higher sugar contents but lower total phenolics, anthocyanins, and ascorbic acid contents (Kelly et al., 2016). Higher anthocyanin content contributes to the 'Florida Radiance' deeper red color than a lighter red-orangish 'Sweet Sensation'® 'Florida127'. 'Sweet Sensation'® 'Florida127' is also a softer fruit than other strawberry cultivars (Kelly et al., 2016), and thus eventually more susceptible to bruising due to rainfall or transportation.

The main objective of this study was to investigate the biochemical changes on 'Florida Radiance' and 'Sweet Sensation'® 'Florida127' strawberry cultivars during postharvest storage at both stress and optimal temperatures. The objective was accomplished by choosing three storage temperatures based on data collected from our previous research (Kelly et al., 2019). Where strawberries were shipped to stores, 8 to 10°C was a standard temperature, and 20°C was typically found during transportation to the consumer. However, 10°C rather than 8°C was chosen for this study because we previously observed a more significant difference in strawberry postharvest behavior during storage at 10 °C compared to 1°C.

Materials and Methods

Plant Material

The two strawberry cultivars used for this research were 'Florida Radiance' and Sweet Sensation'® 'Florida127' (hereafter referred to as Radiance and Sensation, respectively). Both cultivars were harvested from fields at the University of Florida Gulf Coast Research and Education Center (GCREC) in Wimauma,

Florida. Samples were harvested three times during the 2018 season (January - first harvest, February - second harvest, and March - third harvest), transported within one hour of harvest to the University of South Florida Food Quality Laboratory in Tampa, then sorted into clamshell replicates. For the first harvest, two flats of Radiance and one flat of Sensation were collected, containing ~300 and ~120 individual fruits, respectively. For the second and third harvests, two flats of Radiance and three flats of Sensation having ~300 and ~450 individual fruits, respectively, were harvested. For the experimental set-up, 15 fruits per clamshell replicate (21 clamshells total; 3 replicates for each day and temperature) were used for Radiance. For Sensation, 15 fruits per clamshell replicate were used for the second and third harvests; however, for the first harvest, due to pre-harvest water damage on the fruit from rainfall, only 6 fruits were used per clamshell/replicate.

Storage Conditions

The strawberry samples were stored at three different temperatures: 1 ± 0.2°C and 65.0 ± 0.5% RH (VPD = 0.23 KPa), 10 ± 0.7°C and 78.0 ± 0.5% RH (VPD = 0.27 kPa), 20 ± 0.9°C and 71.0 ± 0.1°C (VPD = 0.68 kPa) for up to seven days inside Forma Environmental Chambers (Model 3940 series, Thermo Electron Corporation, Ohio, USA). The three temperatures utilized for storage simulated three different real-world strawberry storage environments: optimum storage at 1°C, transport at 10°C, and temperature stress at 20°C (e.g., when strawberries are left unrefrigerated on the consumer's countertop). Strawberries were stored for 7 days at 1°C and 10°C, whereas due to extreme decay and mold growth during storage at 20°C, strawberry samples were only kept for three days.

Respiration Rate

The measurement of respiration was used to provide information about the metabolic activity of the different strawberry cultivars. The respiration rate was measured using a static system where strawberries were placed in a sealed container (Fig. 5). The accumulation of carbon dioxide or oxygen depletion in the surrounding atmosphere was measured over time using a CheckPoint handheld gas analyzer (Dansensor, Mocon Inc., MN, USA). Respiration rate was recorded on the day of harvest (day 0) for all three different harvests. The samples were probed a total of 5 times (including initial readings) over one hour.

Figure 5. Respiration Rate Set-up. Experimental set-up for measuring respiration rate of fresh strawberries. The jars have an airtight seal, and a needle connected to the meter is inserted into the jars through the rubber valve at the top of the lids.

Weight Loss and Moisture Content

The weight loss of each strawberry cultivar was determined by weighing the samples at harvest and every day for 3 or 7 days, depending on the temperature. Clamshell replicates contained 15 individual fruits, except for Sensation in the first harvest, where clamshells had 6 fruits per replicate. This difference in sampling size was due to severe water damage to the fruits in the field from rain and less available fruit. The moisture content of the samples was determined by drying three weighed aliquots of pureed strawberry tissue at 80°C for 24 hours until weight stabilized. Moisture content measurements were performed on the day of harvest. Weight loss and moisture content data were necessary to allow for expression in dry weight, which takes into account water loss during storage. The following formula was used for water loss corrections: [chemical components (fresh weight) × 100 g / strawberry dry weight + weight loss during storage (g)].

Total and Individual Sugar Contents

Total sugar content analysis was conducted using a Hitachi HPLC system with a refractive index detector and a 300 mm × 8 mm Shodex SP0810 column (Shodex, Colorado Springs, CO) with an SP-G guard column (2 mm x 4 mm) as described by Cayo et al. (2016). The mobile phase delivery rate was set at 0.8 ml/1 min. Standards including sucrose, glucose, and fructose were used to identify retention times for the three sugars. After comparing retention time with the standards, the peaks were identified. The total

sugar amount in the strawberry samples was quantified using calibration curves obtained from different standard concentrations: (2, 4, 6, 10, and 20 $mg \cdot mL^{-1}$).

Ascorbic Acid Content

The determination of total ascorbic acid content was performed using a Hitachi LaChromUltra UHPLC system equipped with a diode array detector and LaCHromUltra C18 2 µm column (2 × 50 mm) (Hitachi, Ltd., Tokyo, Japan) as described by Nunes and Dea (2016). Two grams of pureed sample was mixed with 20 ml cold metaphosphoric acid mixture and centrifuged for 20 minutes at 4700 rpm within a temperature of 4°C. The supernatant was then filtered through a grade 2 Whatman filter paper and filtered through a 0.22 µm filter before HPLC analysis. The analysis was performed at a flow rate of 1 ml/1 min with detection of 254 nm. Mobile phase was comprised of potassium phosphate monobasic (KH2PO4, 0.5 %, w/v) at a pH of 2.5 with metaphosphoric acid (HPO3, 0.1 %, w/v). After comparing retention time with the ascorbic acid standard, the peak was identified. The total ascorbic acid content was quantified by plotting sample concentrations along a standard curve obtained from different concentrations of ascorbic acid standards (10, 20, 30, 50, 100, 150, 200, and 300 $\mu g \cdot mL^{-1}$).

Total Phenolic Content

Total phenolic content was determined using the Folin-Ciocalteau (F-C) assay described by Nunes et al. (Nunes et al., 2005). This assay uses Folin-Ciocalteau's phenol reagent as an oxidizing agent to reduce tyrosine and tryptophan residues resulting in blue color with a maximum absorbance at 765 nm (Sánchez-Rangel et al., 2013). The phenolic compounds within the sample interact with the F-C reagent at a pH of ~10, which was achieved by adding a sodium carbonate solution. The absorbance level is proportional to the concentration of phenolic compounds within the sample (Sánchez-Rangel et al., 2013). To obtain juice, pureed fruits were centrifuged for 20 minutes at 4500 rpm within a refrigerated centrifuge (4ºC). The light-colored supernatant was filtered through cheesecloth into 50 ml centrifuge tubes. Then 0.5 g of supernatant was diluted in 9.5 ml of ultrapure deionized water. Each sample replicate was evaluated in triplicate to account for error. Five mL of diluted F-C reagent (1:9 reagent to water dilution) was added to each diluted sample and vortexed for 5 seconds. Between 30 seconds and 8 minutes after adding the F-C reagent, 4 ml of sodium carbonate (0.71 M) solution was added, and each sample was vortexed for 5 seconds. The samples were incubated at 30ºC for 1 hour then chilled on ice for 30 minutes. The absorbance was read

at 765 nm on a microplate reader and then plotted on a standard curve to determine the concentration of total soluble phenolic compounds. Gallic acid was used to create the four standards for this assay. The standard concentrations were comprised of 0.04 mg/ml, 0.08 mg/ml, 0.12 mg/ml, and 0.16 mg/ml. Total phenolic content was reported on a dry weight basis to compensate for water loss during storage.

Anthocyanin Content

The concentration of anthocyanin contents was determined using the method described by Nunes et al. (Nunes et al., 2005). Two grams of pureed fruit were mixed with 18 ml 0.5% (v/v) HCL in methanol in foil-covered glass jars. The pigments were extracted for 1 hour at 4ºC in the dark then filtered through a double layer of tissue. The samples were then read at 520 nm absorbance to determine the total anthocyanin contents. The anthocyanin pigments are light-sensitive; therefore, the entire process was performed under low light conditions. Anthocyanin contents were reported on a dry weight basis to compensate for water loss during storage.

Statistical Analysis

The Statistical Analysis System computer package (SAS Institute, Inc., 2004) was used to analyze the data from these experiments. The data were analyzed via three-way ANOVA analysis with cultivar, storage temperature, and storage time as the independent variables. Statistical analysis showed a significant difference between harvests leading to separate analysis for each harvest. Significant differences between treatments were detected using Tukey's Studentized Range (HSD) test at the 5% significance level.

Results and Discussion

Respiration Rate

Radiance consistently showed a higher respiration rate (RR) than Sensation over all three harvests (Fig. 6). In a previous study, Chang and Brecht (2020) showed that Sensation strawberries had a higher RR than Radiance. Further, the authors reported that bruising significantly intensified RR, with Radiance strawberries showing higher RR than Sensation after bruising. Results from our study are in agreement with results from Chang and Brecht (2020). Strawberries from the first harvest had a higher RR rate than those from the second and third harvests, regardless of the cultivars (Fig. 6). However, Radiance had consistently higher RR than Sensation.

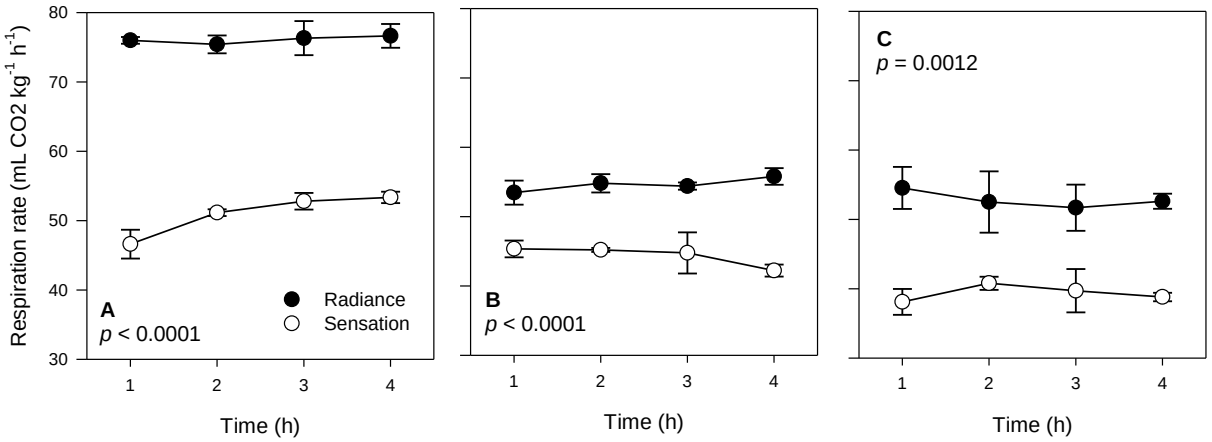

Figure 6. Respiration Rate. Respiration rate for strawberry Radiance (R) and Sensation (S) during storage for 3 days at 20 °C and 7 days at 1°C and 10°C. (A) first harvest, (B) second harvest, and (C) third harvest. Each data point is the average of three replicates containing 10 fruits each.

Higher RR in the first harvest could have resulted from water damage to the fruit due to higher rainfall observed during the first harvest in January (Table 1). Strawberries are generally delicate fruits frequently showing preharvest damage due to adverse environmental conditions. Even just the energy from the raindrop hitting a softer strawberry fruit can lead to bruising (Herrington et al., 2011). Another factor to rain damage is the fruit's surface interacting with this “free water,” leading to potential cell breakdown. The more turgid the fruit's flesh, the lesser the impact of free water damage (Herrington et al., 2011). The link between the turgidity factor and strawberry RR has been documented in wheat. Reintroducing moisture to the crop would increase the respiration rate and boost the metabolism (Rotz, 1994). The higher amounts of rain in the first harvest might have contributed to bruising and water soaking of the fruit resulting in increased respiration rate, particularly in Radiance (Fig. 6).

Table 1. Weather conditions at harvest in the 2018 strawberry season.[a]

	Temperature (°C)			Rainfall)
Harvest date	Max	Min	AVG	(mm)
Jan 29	22.2	14.4	19.4	91.4
Feb 19	30.6	20.6	25.5	0
March 5	23.3	10	16.8	0

Max = maximum; Min = minimum; AVG = average.
[a] Source: Florida Automated Weather Network (https://fawn.ifas.ufl.edu/data/reports).

Weight Loss

Weight loss of strawberries increased during storage, regardless of the harvest, cultivar, or storage temperature (Fig. 7). However, weight loss in strawberries from the first harvest was significantly higher than fruit from the second and third harvests. These results were somehow expected as the first harvest was the only amongst three harvests where strawberries experienced water damage from rainfall (Table 1). It has been previously reported that due to the delicate structure of strawberry fruit, excess rainfall during fruit development in the field can lead to cellular breakdown and water-soaked appearance (Kelly et al., 2016), which was seen in both Sensation and Radiance harvested in January. Overall, Sensation showed a more significant weight loss over time than Radiance in all harvests (Fig. 7). On day 3, fruit stored at 20°C had the most significant weight loss for both cultivars and harvests between the three different storage temperatures. Although after seven days of storage there was a difference between the weight loss of Radiance and Sensation, regardless of the storage temperature, there was no significant difference between the weight loss of strawberry samples stored at 1°C or 10°C for either strawberry cultivar or time of harvest (Fig. 7). These results could be due to the higher RH measured at 10 °C compared to the RH measured at 1 °C (78 and 65% RH, respectively). Besides, calculations of VPD showed that VPD for the strawberry samples stored at 10°C was very close to the RH for those held at 1°C (0.27 and 0.23 kPa, respectively). The rate of weight loss has been previously associated with VPD, based on the principle that transpiration rate is more dependent on VPD than on RH. Ktenioudaki et al. (2021) reported that in blueberry, when VPD was greater than 0.4 kPa, weight loss was higher than 6 to 7% and when VPD values were greater than 1 kPa, weight loss would increase to more than 8% (Ktenioudaki et al., 2021). A high VPD (i.e., greater than 1 kPa) means that the air surrounding the fruit can still hold a large amount of water, creating a great gradient between fruit (saturated with water) and the atmosphere, causing fruit to transpire more and thus losing more water. This concept explains why in our study, strawberries stored at 20°C (VPD = 0.68 kPa) lost significantly more moisture (i.e., higher weight loss) compared to those held at 1°C or 10°C (VPDs = 0.23 and 0.27, respectively) (Fig. 7). Sousa-Gallagher et al. (2013) also reported that in fruit stored at a lower RH, the water vapor pressure deficit could increase outside the fruit leading to an increase in transpiration. The same study also showed that the greater the percent RH, the smaller the water loss due to transpiration (Sousa-Gallagher et al., 2013). Although in this study, the authors used a temperature

higher than 1°C, their results also indicated a minimal difference in weight loss between the samples stored at 5°C and 10°C and the same RH. In our study, the greater RH measured at 10°C than at 1°C, resulted in similar VPDs and thus less transpiration to occur at 10°C. Others have also suggested that differences in the morphological characteristics between genotypes (e.g., the thickness of the cuticle and achene and stomata density) may also contribute to differences in weight loss (Cayo et al., 2016). Another study showed that excessive weight loss results in overall strawberry quality deterioration. Strawberries stored at 20°C attaining a moderate to severe softening rate after approximately 2.5 days, which corresponded to a weight loss of 2.5%, while shriveling of the fruit and dryness of the calyx became evident when weight loss attained 3.0%. Overall, moisture loss can cause subtle quality changes in appearance. However, when the critical moisture loss threshold is reached, more obvious negative changes in turgidity, firmness, discoloration, flavor, and nutritional value occurs (Nunes & Emond, 2007).

Total and Individual Sugar Contents

Total sugars, sucrose, glucose, and fructose, the main sugars found in strawberries, decreased during storage at all temperatures (Figs. 8-11). In general, sucrose contributes the least to the total sugar content in strawberries, whereas glucose and fructose concentrations are about 1:1 ratio (Cordenunsi et al., 2002). Overall, Sensation had higher total and individual sugar contents than Radiance (Fig. 8). However, the first harvest, as shown, was the outlier to this trend, whereas, at harvest, there was no significant difference in the total sugar content between cultivars (Fig. 8A). Strawberries from the second harvest tended to have slightly lower total sugar content on the day of harvest, possibly due to higher field temperatures than the other harvests (Table 1). During storage, in the first harvest, there was an average decrease of 63% in total sugar content from day 0 to day 3. The most significant change was observed in the samples exposed to 20°C (64% and 73% decrease for Radiance and Sensation, respectively). There was little to no significant change in total sugar content from day 3 to day 7 for either cultivar stored at 1°C and 10°C. Weight loss also has been shown to have a significant association with concentrations of all biochemical compounds within the strawberry fruit (Cayo et al., 2016). Cayo et al. (2016) discussed the differences between strawberry Florida cultivars regarding sugar content and how the total sugar content can be affected depending on the harvest date during the season. As for individual sugars, Sensation showed consistently lower sucrose but higher glucose and fructose contents than Radiance

(Figs.9-11). Radiance had a greater amount of sucrose, whereas, by day 7, Sensation had negligible amounts of the same sugar (Fig. 9). Higher sugar contents in Sensation, mainly glucose and fructose, may contribute to the sweeter flavor of the fruit compared to Radiance (data not shown). In previous studies, Whitaker et al. (2015) also showed that Sensation had higher sweetness ratings when compared to Radiance. Others have also reported that the sugar content of strawberries varied between cultivars and harvest time throughout the season and significantly decreased during storage (Cayo et al., 2016; Ferreyra et al., 2007; Montero et al., 1996). Overall, Sensation consistently maintained higher amounts of the two monosaccharides than Radiance. Sucrose, the table sugar, is seen as the standard for sweetness comparisons to other metabolically active sugars and sugar substitutes (Wiebe et al., 2011). According to Wiebe et al. (2011), fructose has a sweetness rating of 1-2 compared to sucrose, whereas glucose has a 0.5-1. While both have the comparison rating of 1, fructose is generally sweeter than sucrose, whereas glucose is less sweet (Wiebe et al., 2011). Sucrose is also the sugar that degrades at a more significant rate after the fruit reaches peak ripeness than glucose and fructose (Ferreyra et al., 2007; Montero et al., 1996). This understanding of sugar biochemistry and previous studies show that a sharp decrease in sucrose content in both cultivars from day 0 to day 3 was expected (Fig. 9). Furthermore, there was little to no significant difference in individual sugar contents between strawberry samples stored at 1°C and the samples stored at 10°C for either 3 or 7 days (Figs. 9-11). However, the levels of individual sugars in fruit stored at 20°C were significantly lower than at the other two temperatures (Figs. 9-11). In the first harvest, there was a greater significant decrease in sugar content overall (Fig. 8A). As in the first harvest, the fruit may have suffered from rainfall damage, respiration rate increased, leading to an overall increase in the metabolism of strawberry fruit. Besides, Radiance had, across harvests, consistently a higher RR than Sensation, which might have also led to a more dramatic decrease in sugar contents during storage. The increase in the overall rate of metabolic reactions (e.g., glycolysis) with increased storage time temperature might also contribute to the breakdown of sugars, depleting fruit reserves.

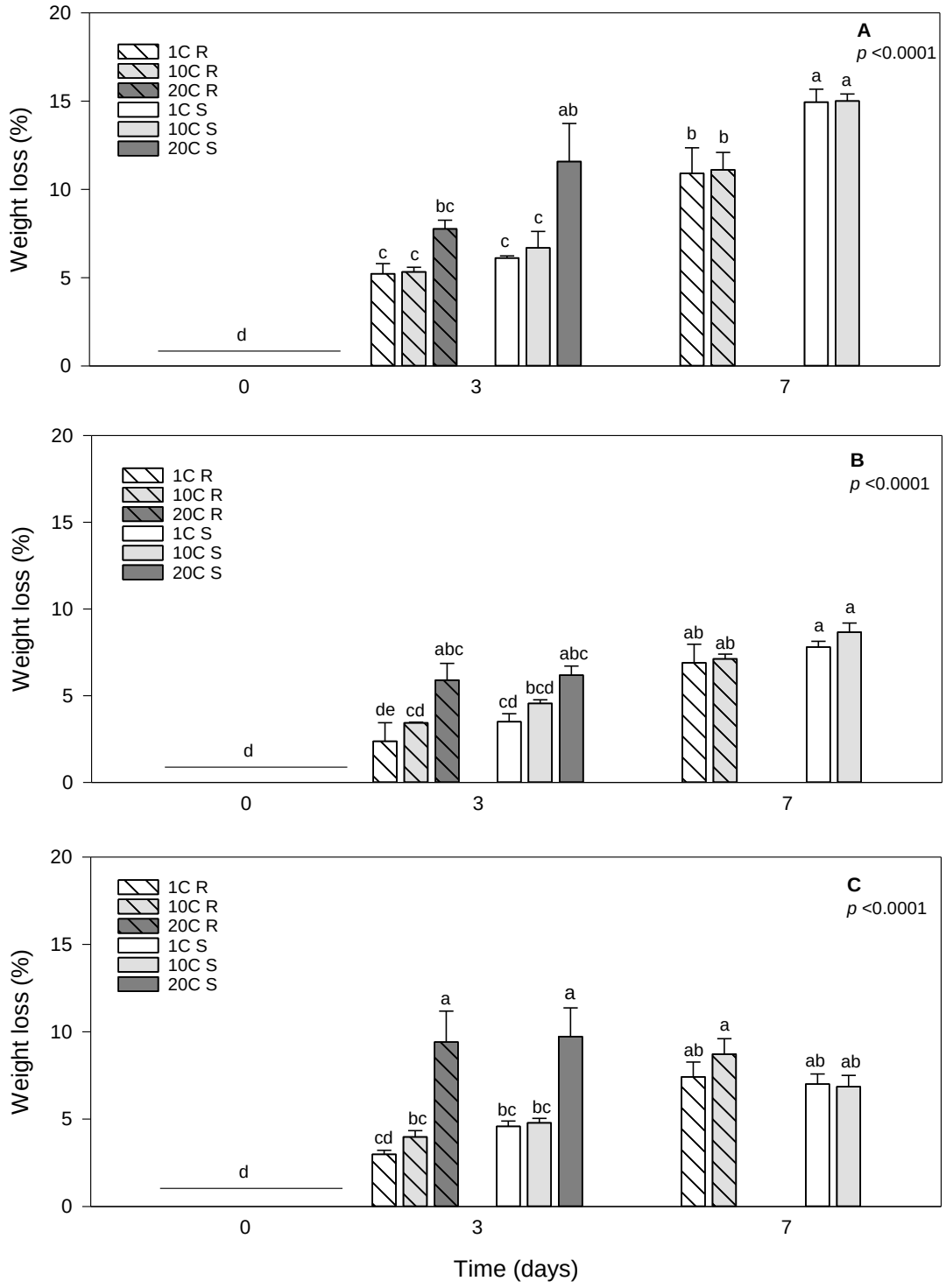

Figure 7. Weight Loss for Radiance and Sensation. Weight loss of strawberry Radiance (R) and Sensation (S) during storage for 3 days at 20 °C and 7 days at 1°C and 10°C. (A) first harvest, (B) second harvest, and (C) third harvest. Each bar represents three clamshell replicates containing 6 (harvest 1) or 15 (harvests 2 and 3) individual fruits each. Bars with the same letter(s) are considered not statistically significant.

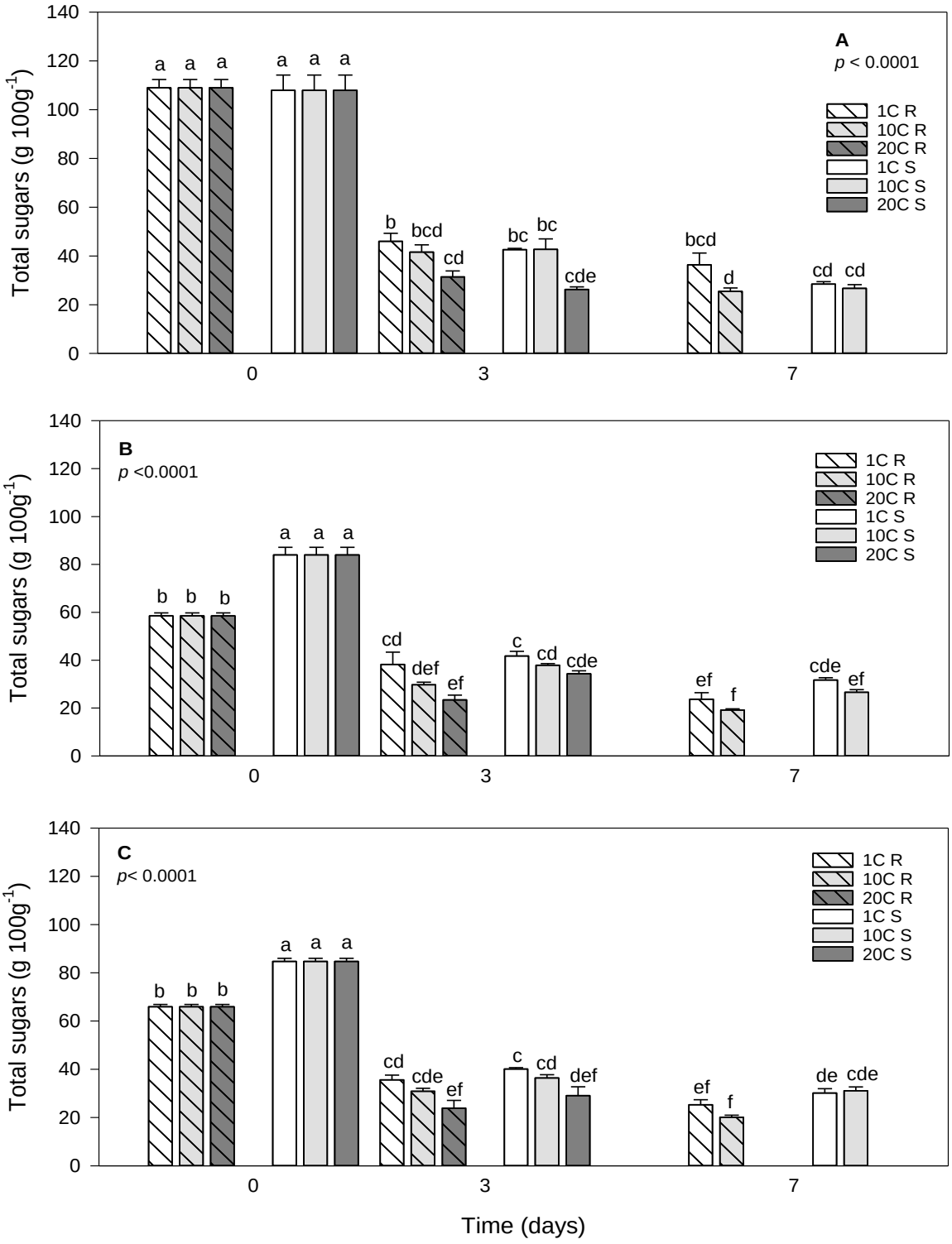

Figure 8. Total Sugar Content for Radiance and Sensation. Total sugar content of strawberry Radiance (R) and Sensation (S) during storage for 3 days at 20 °C and 7 days at 1°C and 10°C. (A) first harvest, (B) second harvest, and (C) third harvest. Each bar represents three clamshell replicates containing 6 (harvest 1) or 15 (harvests 2 and 3) individual fruits each. Bars with the same letter(s) are considered not statistically significant.

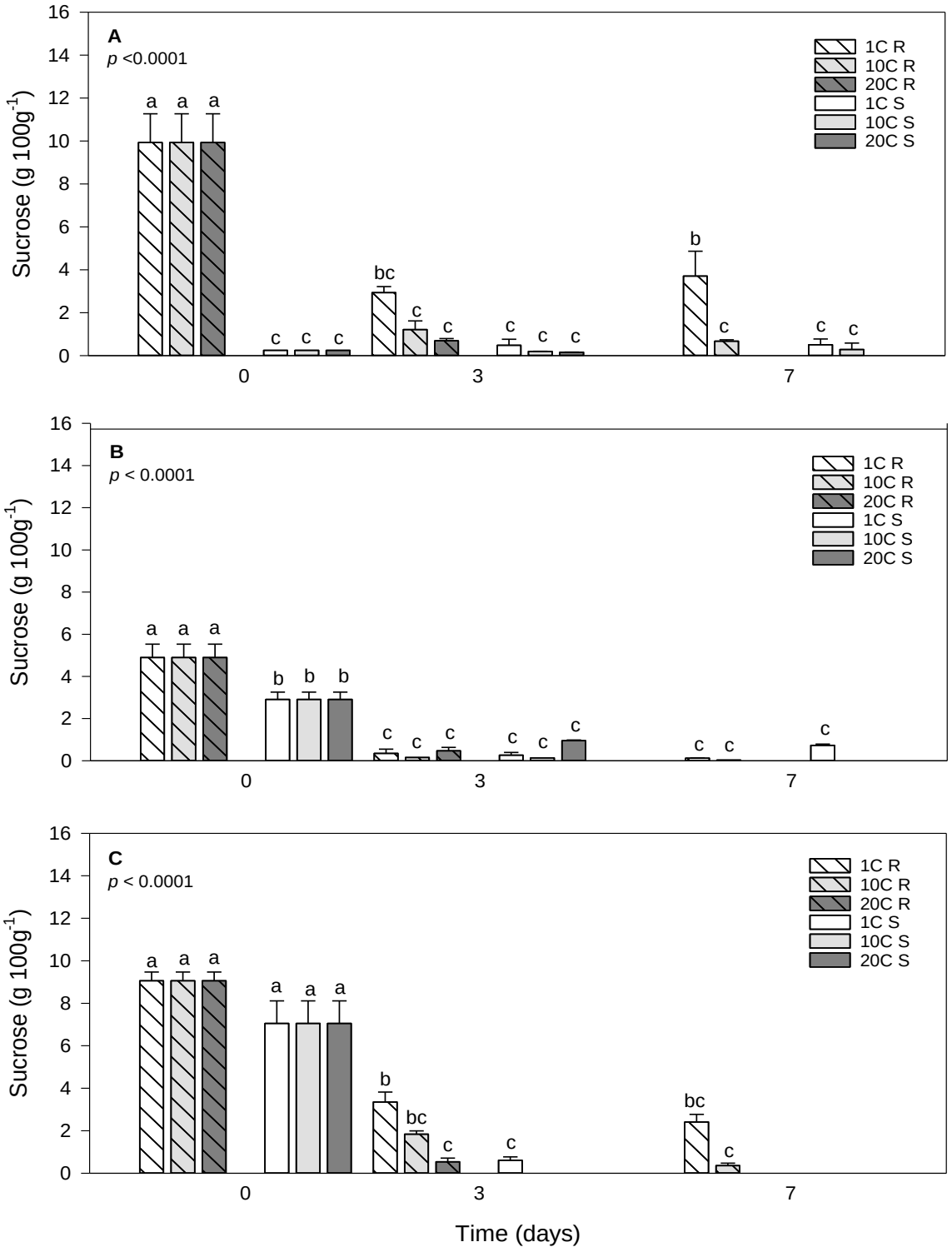

Figure 9. Sucrose Content for Radiance and Sensation. Sucrose content of strawberry Radiance (R) and Sensation (S) during storage for 3 days at 20 °C and 7 days at 1°C and 10°C. (A) first harvest, (B) second harvest, and (C) third harvest. Each bar represents three clamshell replicates containing 6 (harvest 1) or 15 (harvests 2 and 3) individual fruits each. Bars with the same letter(s) are considered not statistically significant.

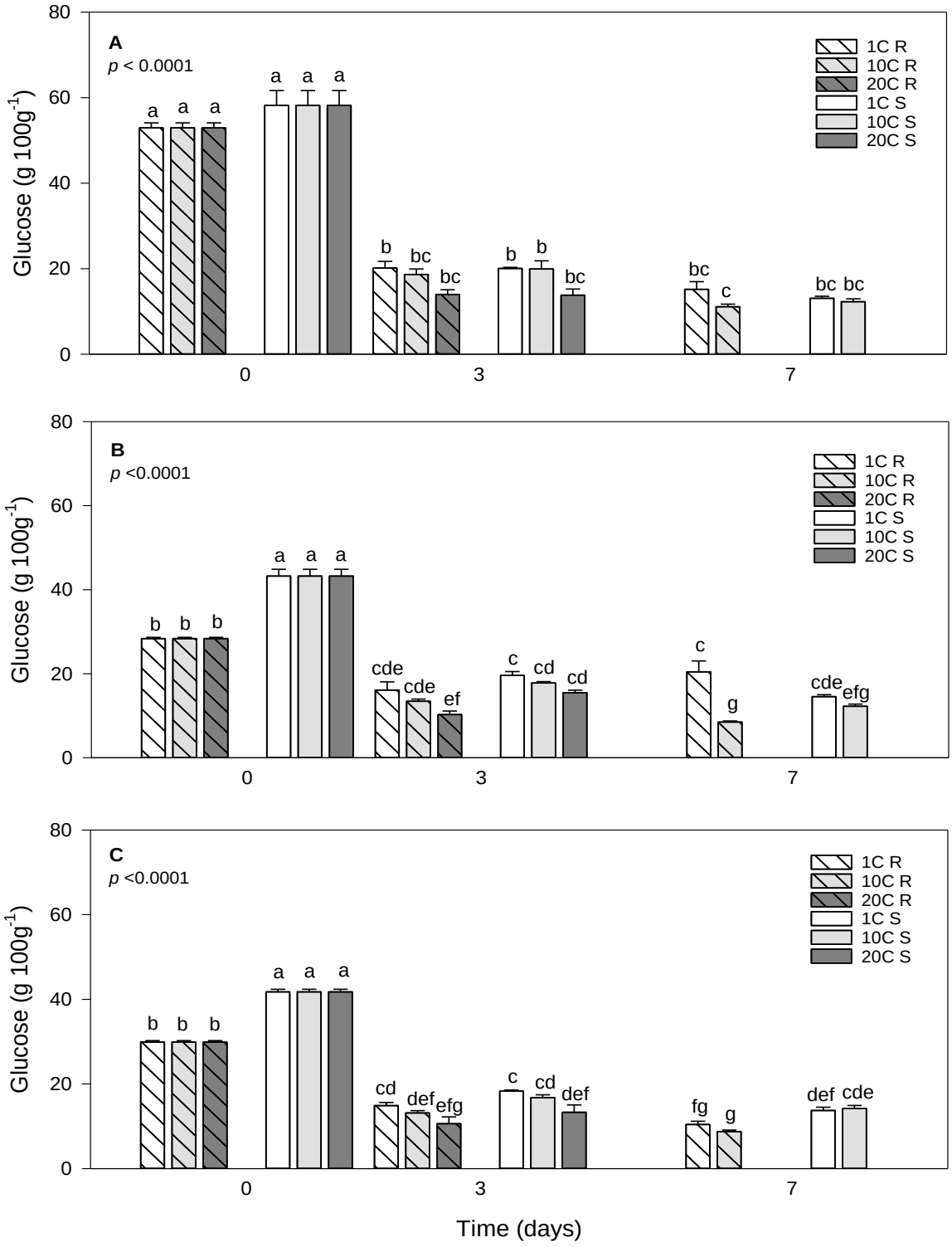

Figure 10. Glucose Content for Radiance and Sensation. Glucose content of strawberry Radiance (R) and Sensation (S) during storage for 3 days at 20 °C and 7 days at 1°C and 10°C. (A) first harvest, (B) second harvest, and (C) third harvest. Each bar represents three clamshell replicates containing 6 (harvest 1) or 15 (harvests 2 and 3) individual fruits each. Bars with the same letter(s) are considered not statistically significant.

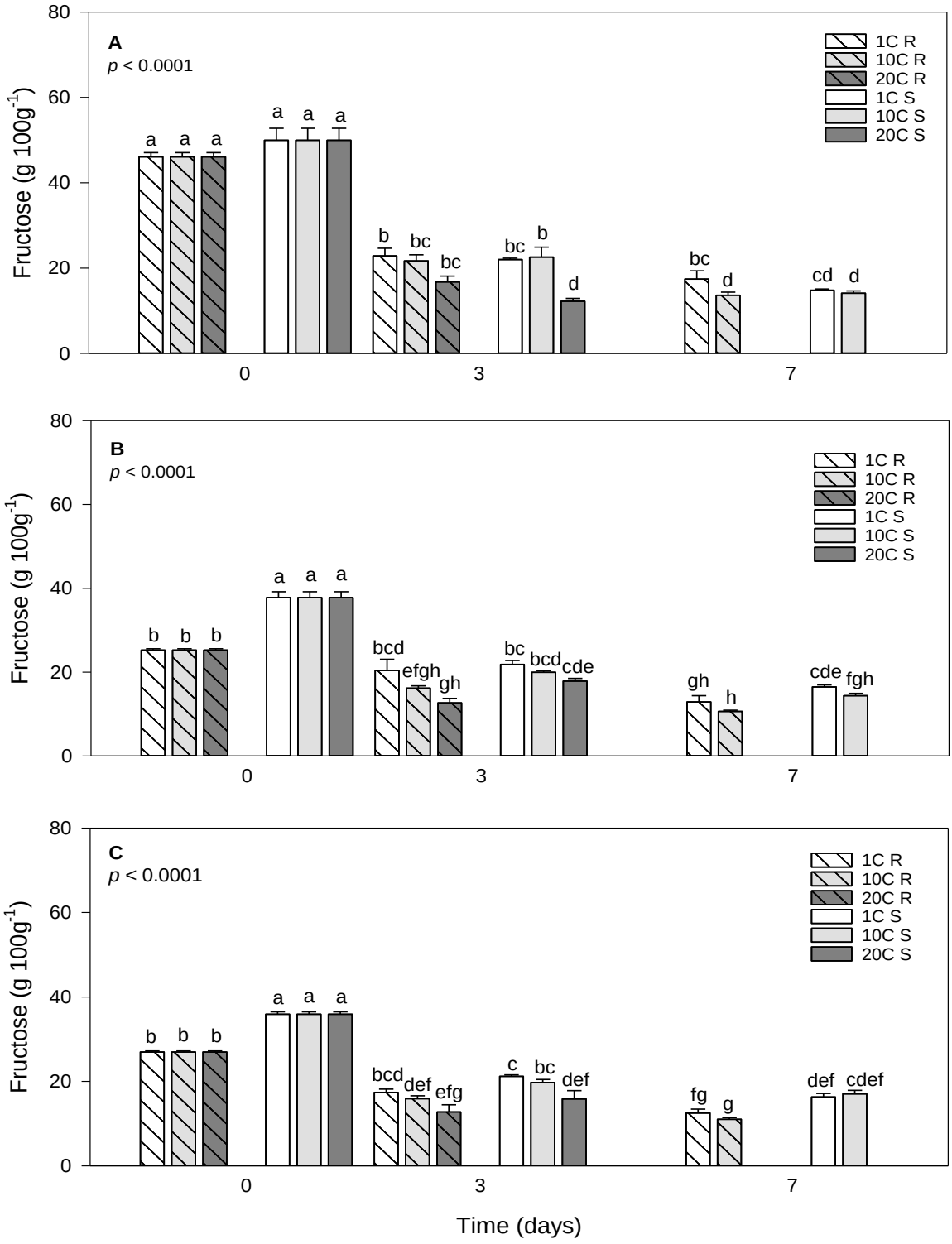

Figure 11. Fructose Content of Radiance and Sensation. Fructose content of strawberry Radiance (R) and Sensation (S) during storage for 3 days at 20 °C and 7 days at 1°C and 10°C. (A) first harvest, (B) second harvest, and (C) third harvest. Each bar represents three clamshell replicates containing 6 (harvest 1) or 15 (harvests 2 and 3) individual fruits each. Bars with the same letter(s) are considered not statistically significant.

Ascorbic Acid Content

Ascorbic acid (AA) accumulates in the fruit during growing; however, it quickly oxidizes when the fruit is detached from the mother plant (Lee & Kader, 2000). Thus, because of the higher rate of oxidation observed for AA compared to other macro or micronutrients, AA constitutes a good marker for the nutritional quality of the food (Lee & Kader, 2000). In general, there was no significant difference in AA contents of Sensation and Radiance strawberries at the time of harvest (Fig. 12). However, a significant decrease in AA contents over time was expected (Fig. 12). There was a considerable difference in AA content in the second harvest between the samples stored for 3 days at 1°C and 10°C (Fig. 12B). On day 7, there was a significant difference between the AA content of strawberries stored at 1°C and 10°C in the first and third harvests (Fig. 12A and 12C). The strawberry samples stored at 20°C showed the most significant decrease in AA than those stored at lower temperatures. However, the fruit from the first harvest had the lowest AA retention by day 7 and had the most significant percent decrease (42.5% and 53.5% for Radiance and Sensation, respectively), possibly due to bruising on the fruit caused by rainfall before harvest. Our results agree with previous studies that showed increased AA oxidation with bruising and cell damage (Lee & Kader, 2000). The most significant decrease in AA content was measured in Sensation (62% decrease) after storage for 7 days at 10°C. On day 3, the most significant reduction in AA content was seen in Sensation held at 20°C (55% decrease from initial values). Others have also shown that AA degrades significantly during postharvest. Kalt (2005) suggested that the decline in AA content could be due to tissue degradation caused by over-ripeness, which agrees with Klein (1987), who stated that nutrient degradation could be accelerated with tissue/cell damage to the fruits. Furthermore, Cayo et al. (2016) showed an overall decrease in AA content during storage regardless of cultivar and Shin et al. (2007) even specifically showed that samples stored at 20°C had a more significant decrease in AA content than samples stored at 0.5°C. Our results agree with those previously published where strawberry samples stored at 20°C showed the greatest decrease in AA content and having the most physical damage due to mold growth and water loss.

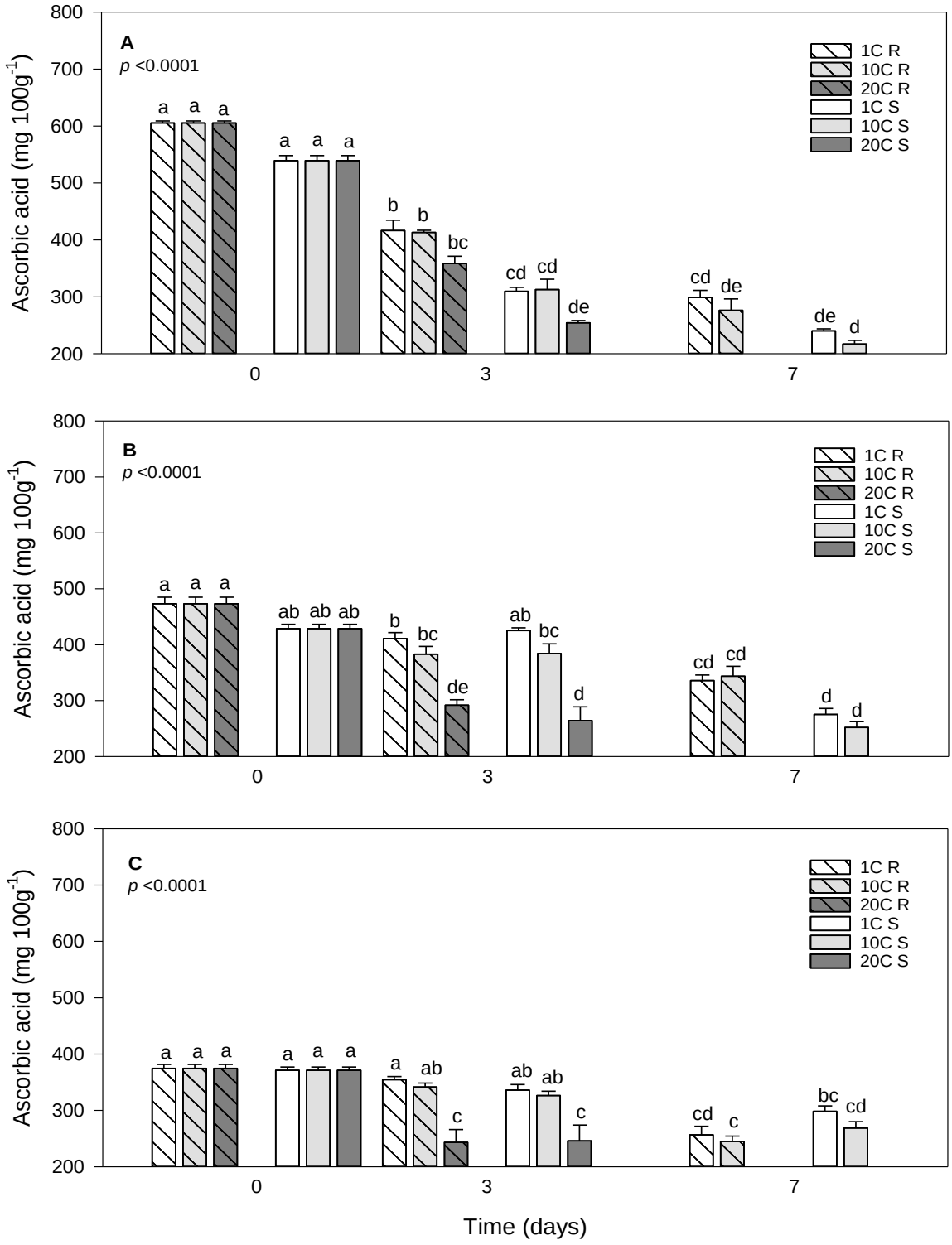

Figure 12. Ascorbic Acid Content of Radiance and Sensation. Ascorbic acid content of strawberry Radiance (R) and Sensation (S) during storage for 3 days at 20 °C and 7 days at 1°C and 10°C. (A) first harvest, (B) second harvest, and (C) third harvest. Each bar represents three clamshell replicates containing 6 (harvest 1) or 15 (harvests 2 and 3) individual fruits each. Bars with the same letter(s) are considered not statistically significant.

Total Phenolic Content

On the day of harvest (day 0), Radiance had, on average, significantly higher total phenolic content (TPC) compared to Sensation, regardless of the harvest date (Fig. 13). There was a decrease in TPC over storage for both cultivars and all three temperatures. However, the strawberry samples stored at 20°C had a more significant decline in TPC than those stored at either 1°C or 10°C. Strawberry Sensation had a greater decrease in TPC for the first and third harvests, with all samples containing less or half of the TPC in Radiance's strawberries from the same treatments (Fig. 13A and 13C). Kelly et al. (2016) showed that Sensation had an overall lesser concentration of TPC than Radiance regardless of harvest time or year.

The unexpected was the lack of significant difference between the strawberry samples stored at 1°C and those held at 10°C. Since 10°C was supposed to be a stress temperature for the strawberries, a more significant change in TPC than the optimum temperature would be expected. However, after looking further into the lack of substantial difference in weight loss between the two temperatures, the absence of significant difference in TPC would likely result. Nunes et al. (2005) discuss how increased water loss was associated with increased loss of total soluble polyphenols. In our study, strawberry samples with the most significant difference in weight loss were stored at 20°C, which also had a more significant decrease in TPC. These results agree with previously published data that water loss during storage is associated with a more substantial reduction in TPC.

Total Anthocyanin Content

Anthocyanins are the major polyphenolic compounds in strawberries and give the fruit its characteristic red color (Aaby et al., 2012). At harvest (day 0), Radiance and Sensation had significantly different total anthocyanin contents (ANC), with Radiance showing higher contents compared to Sensation (Fig. 14).

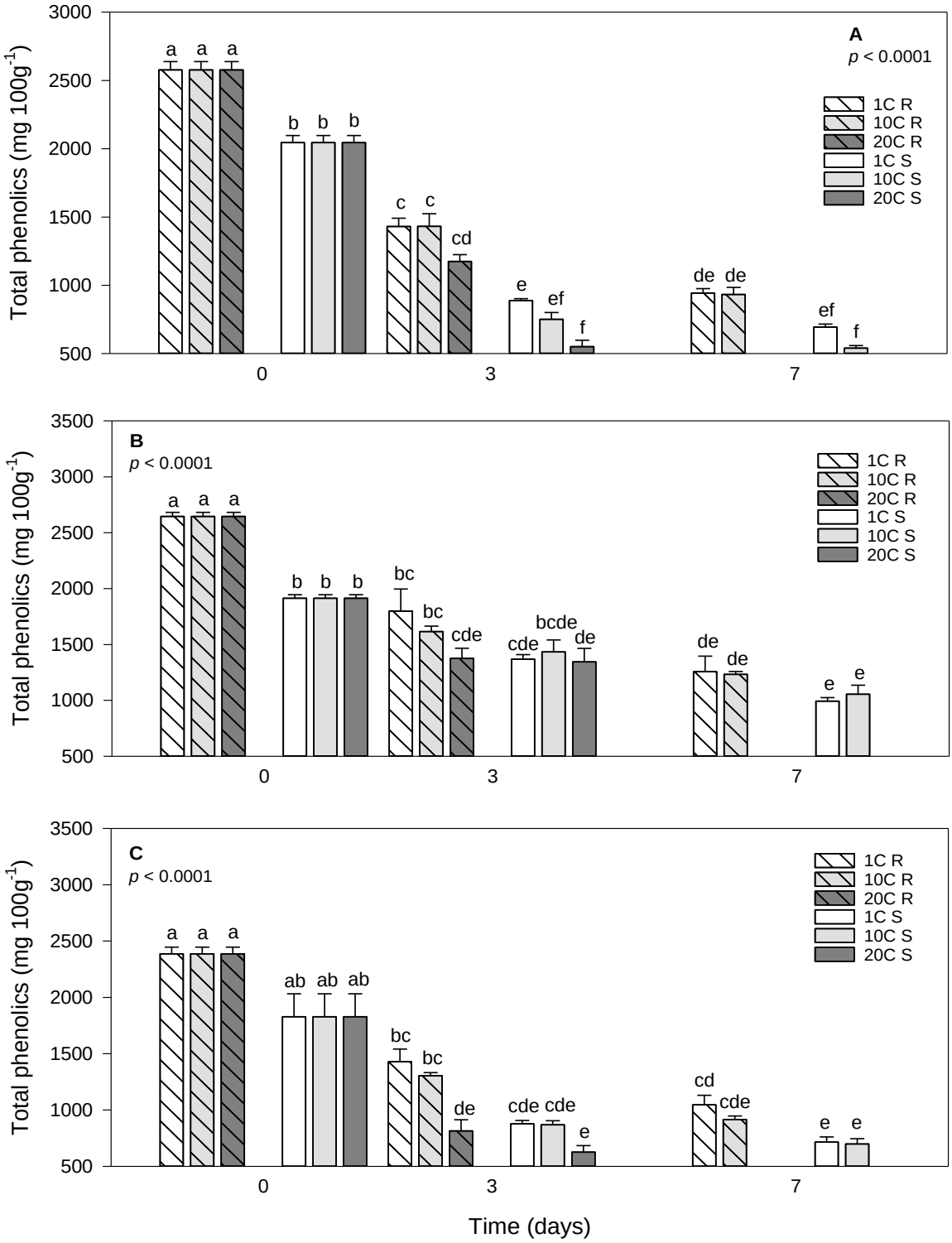

Figure 13. Total Phenolic Content of Radiance and Sensation. Total phenolic content of strawberry Radiance (R) and Sensation (S) during storage for 3 days at 20 °C and 7 days at 1°C and 10°C. (A) first harvest, (B) second harvest, and (C) third harvest. Each bar represents three clamshell replicates containing 6 (harvest 1) or 15 (harvests 2 and 3) individual fruits each. Bars with the same letter(s) are considered not statistically significant.

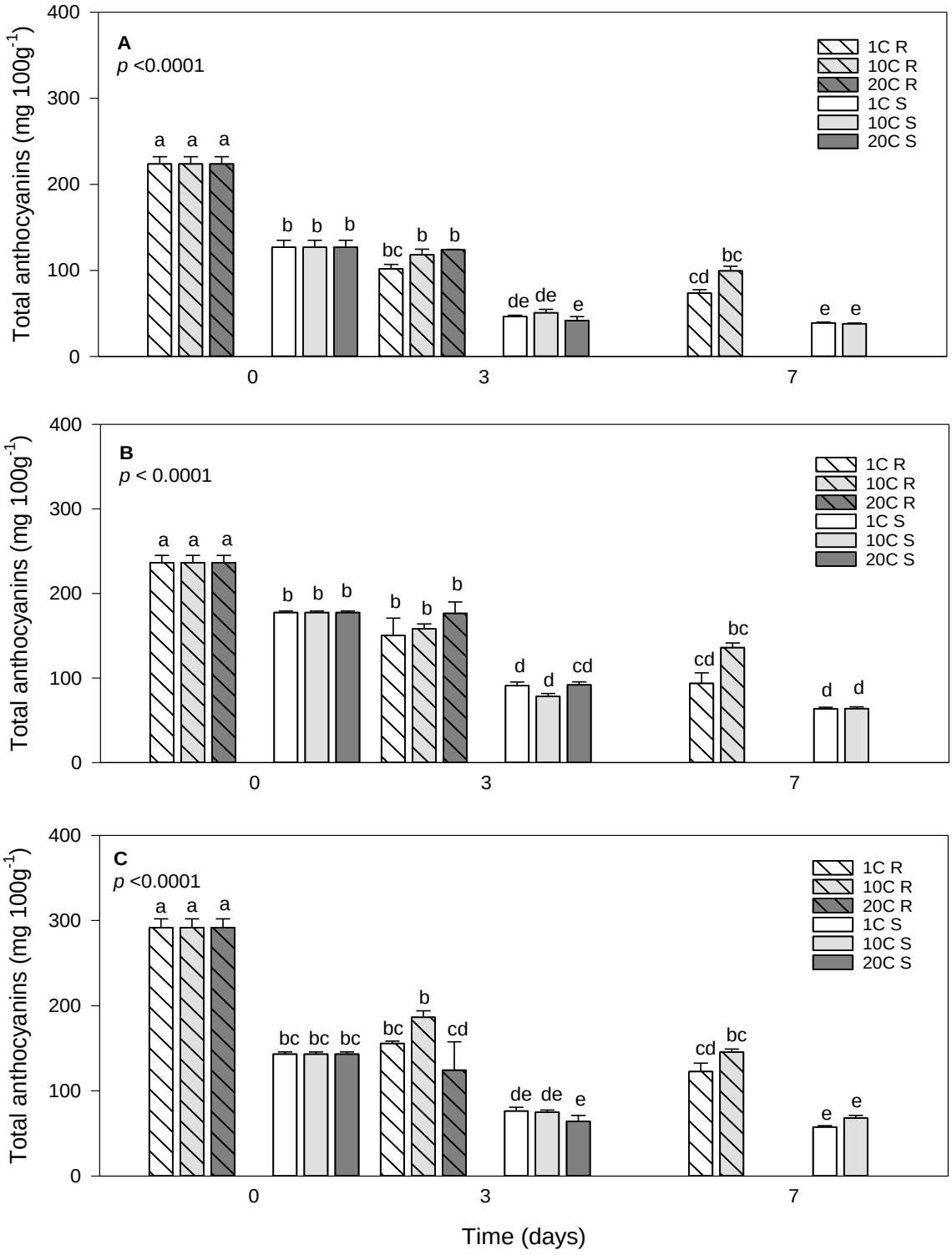

Figure 14. Total Anthocyanin Content of Radiance and Sensation. Total anthocyanin content of strawberry Radiance (R) and Sensation (S) during storage for 3 days at 20 °C and 7 days at 1°C and 10°C. (A) first harvest, (B) second harvest, and (C) third harvest. Each bar represents three clamshell replicates containing 6 (harvest 1) or 15 (harvests 2 and 3) individual fruits each. Bars with the same letter(s) are considered not statistically significant.

Previous studies have shown that Radiance is a significantly “redder” fruit compared to Sensation (Figs. 15 and 16). Specifically, Kelly et al. (2016) reported direct comparisons between Radiance and Sensation strawberry cultivars, showing that Sensation had on average a lower ANC during all harvest times and years. In our study, while there was a consistent decrease in ANC over the storage time, there was a slight increase, although non-significant, in ANC of strawberries stored at higher temperatures. For example, after 3 days of storage, the ANC content of Radiance was slightly higher in fruit stored at 10°C and 20°C compared to fruit stored at 1°C. Few studies also show that ANC may increase when strawberries are stored at higher temperatures (Ayala-Zavala et al., 2004; Shin et al., 2007). However, most of those studies express ANC in terms of fresh weight without accounting for water loss during storage, resulting in most likely in a concentration effect rather than an actual increase. In our study, Radiance strawberries had a consistently higher ANC content when stored at 10°C compared to storage at an optimum temperature of 1°C. These results directly contradict the consistent decrease in TPC observed during storage (Fig. 13). However, anthocyanins are just a portion of the total phenolic content of strawberries. While pelargonidin-3-glucoside may have the overall greatest concentration in most strawberry cultivars, at least 17 total primary polyphenols are seen and studied in strawberry fruits (Kelly, 2018). Therefore, even with an increase in ANC, a decrease in TPC is likely due to the decline in other significant polyphenols.

Studies also showed a decrease in anthocyanin content when strawberries were grown under lower light conditions (Anttonen et al., 2006). It has also been demonstrated in *Arabidopsis thaliana* that chalcone synthase activity, the enzyme which starts the flavonoid synthesis in the phenylpropanoid pathway, increases with increased light exposure as UV rays seems to upregulate the enzyme activity (Cominelli et al., 2008). As natural sun exposure during strawberry growing can be substituted for UV, one can presume that flavonoid synthesis would increase on less cloudy days (i.e., days without rain). Anttonen et al. (2006) showed that strawberries grown under shading conditions (32% reduction in light) tend to have lower ANC than those produced in an open field. In our study, strawberries from the first harvest were exposed to heavy rainfall and decreased UV radiation (Table 1). Thus, that might have contributed to the lower levels of ANC, particularly compared to the third harvest.

Figure 15. Visual comparison of Radiance and Sensation. Visual comparison of Radiance (left) and Sensation (right).

Figure 16. Cross-section of Radiance and Sensation (From Kelly, 2018). Cross-section of Radiance (top) and Sensation (bottom) (From Kelly, 2018).

Conclusions

Overall, there was a significant impact of pre-harvest weather conditions on the biochemical attributes of strawberries, with rainfall during the first harvest affecting the metabolism of the fruit and accelerating biochemical breakdown. Results also showed that exposure of strawberries to 20°C created stress on the fruit, demonstrated by the most significant decrease in all biochemical markers of quality measured, comparatively to storage at 1°C or 10°C. Further research is needed to understand if mild temperature

stress (i.e., 10°C) triggers de novo synthesis of polyphenols or if the increase observed in our study was instead caused by water loss during storage and increased polyphenol concentration. The following chapter (Chapter 4) shows the impact of temperature stress on the individual polyphenol profiles of Radiance and Sensation strawberry cultivars.

CHAPTER FOUR:

IMPACT OF POSTHARVEST TEMPERATURE STRESS ON INDIVIDUAL POLYPHENOL PROFILES OF TWO DIFFERENT STRAWBERRY CULTIVARS

Introduction

Polyphenol compounds are a very diversified class of secondary metabolites produced by all plants. The plant synthesizes these compounds in response to biotic and abiotic stresses such as pests, temperature, draught, and UV radiation (Sarkar & Shetty, 2014). One of the most important roles of polyphenols is to act as an antioxidant within the plant to help combat the reactive oxygen species (ROS) produced by various stresses (Sarkar & Shetty, 2014). Oxidative stress occurs when the abundance of ROS causes cellular damage to the plant (Chaki et al., 2020).

Strawberries are a very important source of polyphenols, namely flavonoids, phenolic acids, and hydrolyzable tannins (Abountiolas & Nunes, 2018; Kelly et al., 2016). Overall, seventeen major polyphenol compounds present in the strawberry fruit are usually grouped into these three classes (Table 2). The flavonoid group comprises the anthocyanidins cyanidin and pelargonidin and the anthocyanins cyanidin 3-glucoside and pelargonidin 3-glucoside. Also, in this group are the flavonols quercetin, kaempferol, quercetin 3-glucoside, kaempferol 3-glucoside, myricetin, and the flavanols catechin, and epicatechin. The phenolic acid group contains *p*-coumaric acid, ferulic acid, caffeic acid, gallic acid, and chlorogenic acid. In strawberries, hydrolyzable tannins are represented by ellagic acid (Giampieri et al., 2012). The primary polyphenols in ripe strawberries, which are also responsible for the fruits' red coloring, are the anthocyanins, particularly pelargonidin-3-glucoside and cyanidin 3-glucoside. However, while they may be the major polyphenols in strawberries, the types and levels of all seventeen polyphenols may differ between cultivars (Kim et al., 2015; Nour et al., 2017). For example, our studies have shown that ‘Florida Radiance’ strawberries have higher total polyphenols and anthocyanins levels than Sweet Sensation’® ‘Florida127’ (Kelly et al., 2016).

The levels of polyphenols in strawberry fruit may also be affected by postharvest conditions, particularly temperature. Several studies have shown the effect of temperature on the levels of total phenolics and anthocyanin contents in strawberry fruit (Ayala-Zavala et al., 2004; Muley & Singhal, 2020; Shin et al., 2007). Besides, in the previous chapter (Chapter 3), results showed that the levels of total phenolics and anthocyanins are greatly influenced by preharvest conditions, type of cultivar, and storage time-temperature combinations. However, very few studies show the effect of postharvest storage conditions on polyphenol profiles of strawberry fruit. Also, to our knowledge, there are no data available on the impact of different storage temperatures on the profiles of major strawberry polyphenols.

Table 2. Major strawberry polyphenols.

Compound Peek Number	Class	Group	Polyphenol	tRa (minutes)	Wavelength (nm)
1	**Flavonoids**	Anthocyanidins	Cyanidin	5.05	520
2			Pelargonidin	5.80	520
3		Anthocyanins	Cyanidin-3-glucoside	3.88	520
4			Pelargonidin-3-glucoside	4.30	520
5		Flavonols	Kaempferol-3-glucoside	6.30	250
6			Kaempferol	9.04	360
7			Quercetin-3-glucoside	5.74	250
8			Quercetin	7.89	250
9			Myricetin	6.63	360
10		Flavanols	Catechin	3.64	280
11			Epicatechin	4.41	280
12	**Phenolic Acids**	Cinnamic Acids	Caffeic acid	4.18	360
13			*p*-Coumaric acid	5.22	280
14			Ferulic acid	5.72	280
15			Gallic acid	1.50	250
16		Hydroxycinnamic esters	Chlorogenic acid	3.74	280
17	**Hydrolyzable Tannins**	Ellagitannins	Ellagic acid	5.28	250

The overall objective of this study was to investigate the differences in polyphenol profiles between two different Florida strawberry cultivars. Further, changes in the polyphenol profile within each cultivar due to postharvest stress (i.e., time-temperature stress) were also evaluated. To accomplish our main objective,

'Florida Radiance' and Sweet Sensation'® 'Florida127' strawberries harvested in January (first harvest) and March (third and last harvest of the season) were chosen for this study. The rationale behind our choice was that fruit from the first harvest experienced preharvest stress due to rainfall which potentially affected the polyphenol profiles. The data from the third harvest showed a more consistent trend than data from the second harvest (Chapter 3). To study the effect of postharvest temperature stress on polyphenol profiles, we chose to hold strawberries for 3 days at 1°C, 10°C, and 20°C because strawberry samples exposed to 20°C for more than 3 days developed objectionable decay.

Materials and Methods

Plant Material

The two strawberry cultivars used for this research were 'Florida Radiance' and Sweet Sensation'® 'Florida127' (hereafter referred to as Radiance and Sensation, respectively). Both cultivars were harvested from fields at the University of Florida Gulf Coast Research and Education Center (GCREC) in Wimauma, Florida. Samples were harvested two times during the 2018 season (January - first harvest and March - third harvest), transported within one hour of harvest to the University of South Florida Food Quality Laboratory in Tampa, then sorted into clamshell replicates. For the first harvest, two flats of Radiance and one flat of Sensation were collected, containing ~300 and ~120 individual fruits, respectively. For the third harvest, two flats of Radiance and three flats of Sensation having ~300 and ~450 individual fruits, respectively, were harvested. For the experimental set-up, 15 fruits per clamshell replicate (21 clamshells total; 3 replicates for each day and temperature) were used for Radiance. For Sensation, 15 fruits per clamshell replicate were used for the third harvests; however, for the first harvest, due to pre-harvest water damage on the fruit from rainfall, only 6 fruits were used per clamshell.

Storage Conditions

The strawberry samples were stored at three different temperatures: 1 ± 0.2°C and 65.0 ± 0.5% RH (VPD = 0.23 KPa), 10 ± 0.7°C and 78.0 ± 0.5% RH (VPD = 0.27 kPa), 20 ± 0.9°C and 71.0 ± 0.1°C (VPD = 0.68 kPa) for up to seven days inside Forma Environmental Chambers (Model 3940 series, Thermo Electron Corporation, Ohio, USA). The three temperatures utilized for storage simulated three different real-world strawberry storage environments: optimum storage at 1°C, transport storage at 10°C, and

temperature stress at 20°C (e.g., when strawberries are left unrefrigerated on the consumer's countertop). Strawberries were stored for 7 days at 1°C and 10°C, whereas due to extreme decay and mold growth at 20°C, strawberry samples were only kept for three days.

Sample Selection for Individual Polyphenol Testing

Following statistical analysis of biochemical data and the results obtained for weight loss and respiration rate (Chapter 3), it was determined that day 7 would not be used for further testing. This decision was taken because, first, the data from the 7-day temperature stress at 20°C was not be accounted for due to excessive decay on the fruit. Secondly, there was no significant difference between the biochemical profiles of strawberries stored for 7 days at 1°C and 10°C. Therefore, it was decided that for further experiments on individual polyphenol profiles, only strawberry samples from days 0 and 3 stored at 1°C, 10°C, and 20°C would be used. Besides, it was also decided that the first and third harvests would be utilized for further polyphenol profile analyzes because results obtained for strawberries from the third harvest had a more consistent trend than data obtained for the second harvest. Usually, the outlier (i.e., first harvest) would have been eliminated; however, since the biochemical quality of strawberries from the first harvest was impacted by rainfall and water damage from uncontrollable weather, further testing could reveal differences in how the fruits reacted to an added pre-harvest stressor (i.e., rainfall).

Polyphenol Extraction

The extraction of the polyphenols was performed as described previously by Abountiolas and Nunes (2018) with minor adjustments such as using fruit puree at the start instead of juice. Triplicates of 5 g of fruit puree were mixed with 15 mL of acetone and homogenized using a Polytron for 1 minute, then sonicated for 10 minutes, and finally filtered through Whatman paper No.4. The filtrate was concentrated to 5 mL in an SPD121P SpeedVac® Concentrator (Thermo Fisher Scientific Inc., Asheville, NC, USA) and finally passed through a classic C18 Sep-Pack cartridge (Waters Technologies Corp., USA). The Sep-Pack cartridge was activated with ~5 mL each methanol followed by ultrapure water and finally, 3% acidified water before passing the concentrated sample. Anthocyanins and other phenolic compounds present were absorbed into the cartridge, while sugars, acids, and other water-soluble compounds were eluted with ~10 mL acidified water. The phenolic compounds were then recovered by passing ~2 mL of methanol

(containing 3% formic acid) through the cartridge. The extracted sample was filtered through a 0.20 µm syringe filter into 2 mL autosampler vials and stored at -30°C until the time of analysis.

Individual Polyphenol Analysis

Analysis of phenolic compounds was conducted using a Hitachi LaChroma Ultra HPLC system coupled with a photodiode array detector (Hitachi, Japan) as according to Kelly (2018) with minor adjustments regarding retention times and dry weight conversions. Samples were injected at 40°C onto a reverse-phase Hypersil Gold C18 column (100 × 2.1 mm; particle size, 1.9 µm) (Thermo Fisher Scientific Inc., USA). The two mobile phases consisted of acidified water containing 0.5% formic acid (mobile phase A) and 0.1% formic acid in acetonitrile (mobile phase B) in an isocratic mixture. The flow rate was 0.3 µL/min, the wavelength detection included 250, 280, 360, and 520 nm, and the sample injection volume was 10 µL. Retention times and spectra were compared with pure standards of 17 compounds (Table 1) (at concentrations of 0.005 mg/mL, 0.01 mg/mL, and 0.1 mg/mL) from different polyphenol classes: flavonoids (cyanidin, pelargonidin, cyanidin 3-glucoside, pelargonidin 3-glucoside, quercetin, kaempferol, quercetin 3-glucoside, kaempferol 3-glucoside, myricetin, catechin, and epicatechin), phenolic acids (p-coumaric acid, ferulic acid, caffeic acid, gallic acid, and chlorogenic acid) and hydrolyzable tannins (ellagic acid). The flavonoids can be further classified into anthocyanidins (cyanidin and pelargonidin), anthocyanins (cyanidin 3-glucoside, and pelargonidin 3-glucoside), flavonols (quercetin, kaempferol, quercetin 3-glucoside, kaempferol 3-glucoside, and myricetin), and flavanols (catechin and epicatechin). Quantification of the individual polyphenols was be based on the concentration of mg/100g fruit in dry weight.

Statistical Analysis

The Statistical Analysis System computer package (SAS Institute, Inc., 2004) was used to analyze the data from these experiments. The data were analyzed via three-way ANOVA analysis with cultivar, storage temperature, and storage time as the independent variables. Statistical analysis showed a significant difference between harvests leading to separate analysis for each harvest. Significant differences between treatments were detected using Tukey's Studentized Range (HSD) test at the 5% significance level.

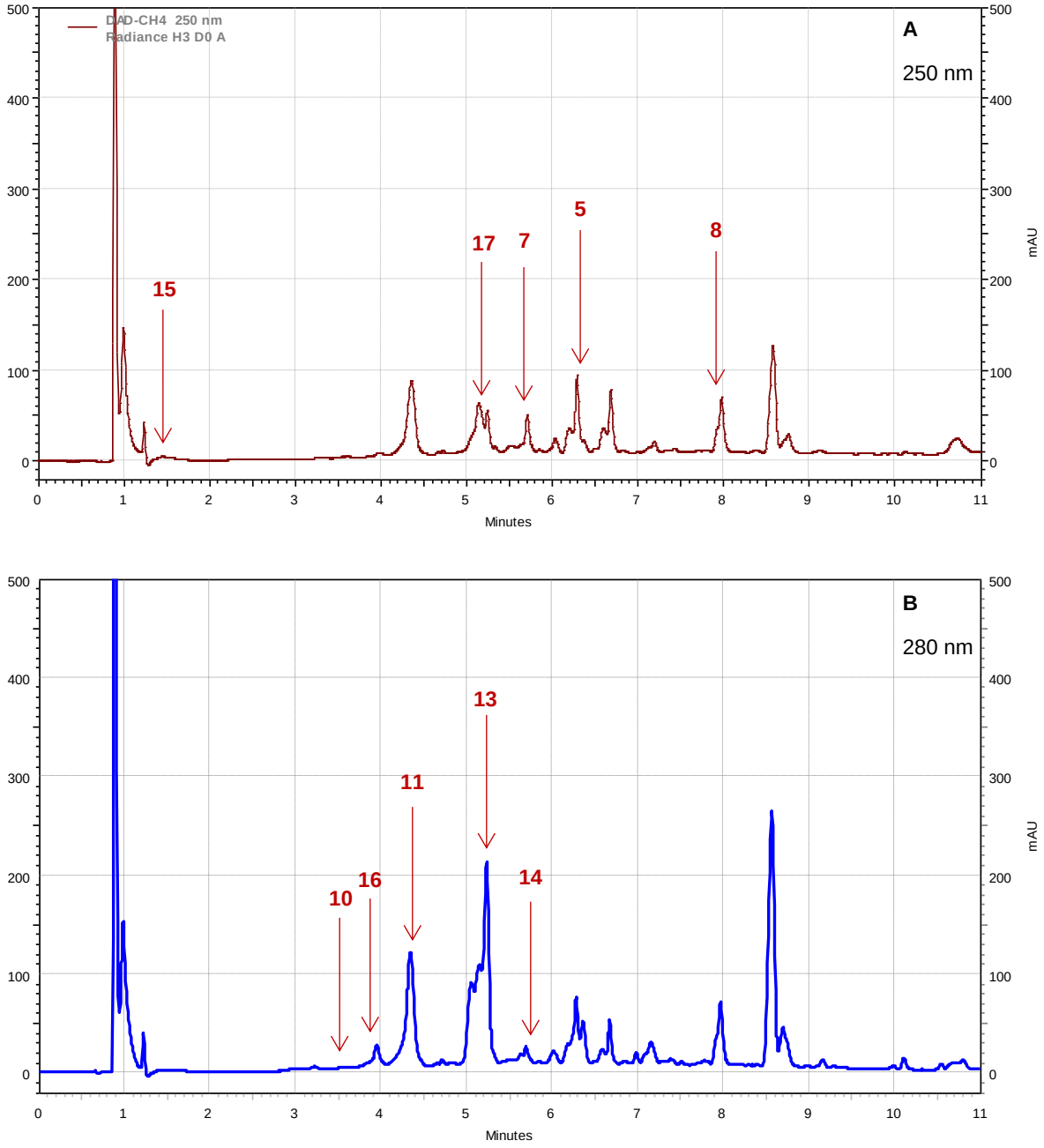

Figure 17. HPLC Chromatograms at 250 and 280nm for Radiance. Example of HPLC chromatograms for individual polyphenols for strawberry Radiance measured at the time of harvest (initial; D0). Wavelengths 250 nm (**A**) and 280 nm (**B**). Peak numbers: 5) kaempferol 3-glucoside, 7) quercetin 3-glucoside, 8) quercetin, 10) catechin, 11) epicatechin, 13) *p*-coumaric acid, 14) ferulic acid, 15) gallic acid, 16) chlorogenic acid, and 17) ellagic acid.

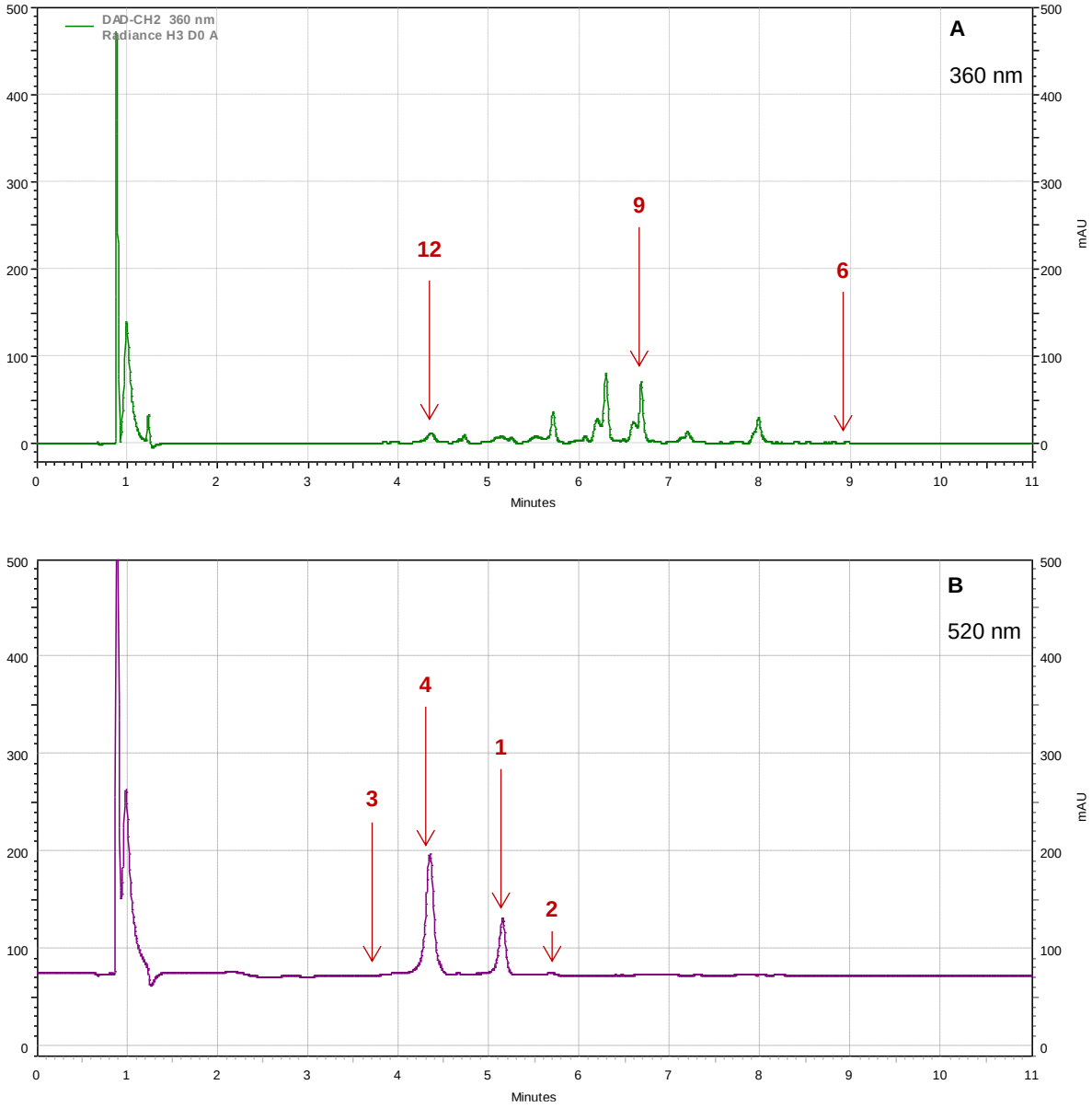

Figure 18. HPLC Chromatograms at 360 and 520nm for Radiance. Example of HPLC chromatograms for individual polyphenols for strawberry Radiance measured at the time of harvest (initial; D0). Wavelengths 360 nm (**A**) and 520 nm (**B**). Peak numbers: 1) cyanidin, 2) pelargonidin, 3) cyanidin-3-glucoside, 4) pelargonidin 3-glucoside, 6) kaempferol, 9) myricetin, and 12) caffeic acid.

Results and Discussion

Individual Polyphenols

The levels of anthocyanins in strawberries are a major determinant of fruit color and are highly correlated with the redness of the skin and flesh (Nunes, 2015). In this study, Radiance showed at the time of harvest (initial) consistently higher concentrations of pelargonidin-3-glucoside than Sensation across the two harvests (Tables 3 and 4). Figures 17 and 18 show examples of HPLC chromatograms for individual polyphenols for strawberry Radiance measured at the time of harvest (initial). These results agree with results obtained from the previous chapter (Chapter 3) and others (Kelly et al., 2016), where Radiance had higher levels of total anthocyanins than Sensation. Overall, in the first harvest, Radiance had an initial higher content of almost all seventeen primary polyphenols, specifically pelargonidin-3-glucoside, epicatechin, kaempferol-3-glucoside, and quercetin compared to Sensation (Table 3). However, there was no significant difference in the kaempferol, catechin, and caffeic acid contents between the two strawberry cultivars (Table 3). Radiance had significantly higher levels of all polyphenols in the third harvest than Sensation, particularly pelargonidin 3-glucoside, quercetin, epicatechin, caffeic acid, p-coumaric acid, and chlorogenic acid (Table 4). Those results were consistent for both harvests; however, for the first harvest, Radiance also had a greater concentration of *p*-coumaric acid and quercetin-3-glucoside at harvest (Table 3). For the third harvest, Radiance had at harvest (initial) a greater concentration of caffeic acid and cyanidin along with pelargonidin-3-glucoside, epicatechin, kaempferol-3-glucoside, and quercetin (Table 4). Strawberry Sensation had a greater concentration of quercetin-3-glucoside in the third harvest (Table 4). Epicatechin was present at the greatest concentration among all seventeen polyphenols, followed by pelargonidin-3-glucoside (Tables 3 and 4). With epicatechin and cyanidin-3-glucoside both being products of cyanidin reduction and glycosylation, respectively (Fischer et al., 2014), it makes sense that while the levels of epicatechin were very high, cyanidin-3-glucoside was non-detectible. Nour et al. (2016) showed a significant difference in quercetin, myricetin, and epicatechin between two strawberry cultivars (‘Magic’ and ‘Premial’). However, another study using five different strawberry cultivars found no significant difference in quercetin content between cultivars and no detectible myricetin (Hernanz et al., 2007).

Kim et al. (2015) showed variability between fourteen strawberry cultivars regarding ellagic acid. Besides, the harvest time also influenced the ellagic acid content of the different strawberry cultivars. In

addition, the harvest time has also been shown previously to affect the anthocyanin content of strawberry cultivars (Kim et al., 2012). Thus, the levels of pelargonidin-3-glucoside and cyanidin-3-glucoside were significantly different between cultivars and years of harvest for the same cultivar (Kim et al., 2015). The specific influence factor seems to be the temperature during the development and harvest of the fruits, with the clusters harvested at hotter points in the season showing more significant levels of anthocyanins (Kim et al., 2012). In our study, the strawberry fruits were from harvests chosen at the beginning and end of the season, presenting different weather conditions (i.e., first harvest average temperature 19.4 °C and 91.4mm rainfall, and third harvest 16.8°C and 0 mm rainfall; Chapter 3 - Table 1) which may be an explanation for the significant differences between polyphenol profiles. Thus, compared to the third harvest, strawberries from the first harvest had lower levels of cyanidin, pelargonidin, pelargonidin 3-glucoside, and cyanidin 3-glucoside amongst other polyphenols, regardless of the cultivar, most likely due to the lower solar radiation (i.e., higher rainfall). It has also been shown by Herrington et al. (2011) that rainfall can significantly impact the strawberry respiration rate (RR) (Herrington et al., 2011). Adhikary et al. (2021) also reported that increased RR is correlated with an increase in polyphenol oxidase (PPO) activity (Adhikary et al., 2021). PPO is a key oxidative enzyme in the phenylpropanoid pathway, and this would explain why the fruits from the first harvest would have a lower initial phenolic content than the fruits from the third harvest. Regarding lower solar radiation or UV light exposure, it has been shown that while UV light exposure can increase the total phenolics and anthocyanins within a strawberry fruit, not all cultivars may show a significant increase in the levels of those biomolecules (Cervantes et al., 2019; Palmieri et al., 2017).

Along with the effect of harvest time and cultivar, the postharvest storage environment also impacted the levels of different polyphenols in the two strawberry cultivars. Thus, there was a significant change in the polyphenol profiles after 3 days (Tables 3 and 4). Zhang et al. (2019) showed not only an increase in total polyphenols in samples stored at higher temperatures (25 °C and 35 °C) but specifically an increase in pelargonidin-3-glucoside (L. Zhang et al., 2019). These findings were also confirmed by the increase in the activity of phenylalanine ammonia lyase (PAL), the regulatory enzyme of the phenylpropanoid pathway, in strawberry samples stored at high temperatures (Zhang et al., 2019).

Table 3. The concentration (mg $100g^{-1}$ of fruit dry weight) of the major individual polyphenols in Radiance and Sensation strawberry cultivars at harvest (initial) and after 3 days at 1°C, 10°C and 20°C (Harvest 1).

Polyphenols	Radiance				Sensation				
	Initial	1°C	10°C	20°C	Initial	1°C	10°C	20°C	p value
Anthocyanidins									
Cyanidin	13.5±0.3 ab [a]	9.0±0.5 b	21.6±6.1 a	8.7±0.7 b	12.1±0.2 ab	7.0±0.2 b	6.9±0.5 b	7.2±1.0 b	0.0040
Pelargonidin	45.9±5.4 a	23.4±1.8 b	39.3±9.5 ab	19.0±0.8 b	34.4±0.4 ab	22.3±2.6 b	20.6±1.6 b	20.3±4.2 b	0.0023
Anthocyanins									
Cyanidin 3-glucoside	ND [b]	ND	ND	ND	ND	ND	ND	ND	ND
Pelargonidin 3-glucoside	169.7±17.8 abc	175.6±33.8 ab	227.3±0.0 a	239.6±45.7 a	64.9±9.8 cd	60.0±8.4 d	59.8±4.8 d	68.5±4.3 bcd	<0.0001
Flavonols									
Kaempferol 3-glucoside	78.2±6.3 a	65.5±8.5 ab	91.9±0.0 a	74.0±10.9 a	58.0±10.0 abc	35.4±9.3 bcd	20.4±5.2 d	27.0±7.3 cd	<0.0001
Kaempferol	12.7±0.5 a	9.7±0.9 ab	10.3±1.0 ab	7.9±1.0 b	13.0±0.6 a	8.6±0.6 b	8.4±0.7 b	7.3±0.3 b	0.0004
Quercetin 3-glucoside	80.2±3.5 a	46.6±5.5 bc	59.6±0.0 ab	52.9±12.4 abc	59.9±8.7 ab	44.0±6.9 bc	25.9±3.3 c	34.1±2.3 bc	0.0014
Quercetin	42.9±4.3 a	24.1±3.0 bcd	32.0±4.3 ab	29.1±6.1 abc	21.0±0.6 bcd	12.6±2.2 cd	7.3±0.9 d	11.1±0.7 d	<0.0001
Myricetin	27.1±1.0 a	19.7±2.0 ab	31.9±7.3 a	20.5±3.8 ab	19.8±0.4 ab	12.0±1.0 b	10.9±1.2 b	11.1±0.9 b	0.0015
Flavanols									
Catechin	16.0±3.6 a	21.7±4.8 a	29.8±0.0 a	19.0±10.4 a	17.5±0.6 a	24.5±2.1 a	17.5±2.0 a	34.6±12.1 a	0.3135
Epicatechin	263.8±30.0 abc	285.0±60.5 ab	376.4±0.0 a	391.6±83.1 a	96.3±12.9 bc	93.3±11.9 bc	80.1±6.6 c	112.5±5.2 bc	<0.0001
Phenolic acids									
Caffeic Acid	32.6±11.4 ab	34.0±4.1 ab	35.1±0.0 ab	35.2±7.4 ab	41.8±7.1 a	12.1±0.0 b	20.1±0.0 ab	13.8±7.2 ab	0.0209
p-Coumaric Acid	40.9±2.6 ab	21.8±3.7 b	124.1±49.8 a	24.3±5.6 b	19.2±1.8 b	6.8±1.2 b	4.2±0.3 b	7.3±0.5 b	0.0051
Ferulic Acid	34.1±1.3 ab	15.4±1.8 b	58.1±18.7 a	19.5±4.1 b	26.1±5.0 ab	15.7±1.8 b	9.9±0.9 b	25.6±11.8 ab	0.0138
Gallic Acid	77.7±14.0 a	21.3±14.5 b	29.3±14.5 ab	21.2±7.9 b	77.4±11.0 a	37.9±5.0 ab	32.2±8.1 ab	30.5±9.0 ab	0.0078
Chlorogenic Acid	27.4±4.9 a	21.8±4.5 a	28.8±0.0 a	36.9±6.3 a	19.8±3.0 a	20.9±3.0 a	18.9±1.7 a	36.3±14.5 a	0.2261
Hydrolyzable tannins									
Ellagic Acid	15.4±2.9 ab	7.8±0.5 c	16.3±3.6 a	9.4±1.8 bc	11.5±0.6 abc	11.7±1.0 abc	7.8±1.3 c	10.7±1.8 abc	0.0490

[a] Letters after averages denote significant differences ($p < 0.05$) between treatments based on Tukey's HSD test; averages followed by the same letter are not significantly different.
[b] ND = non-detected.

Table 4. The concentration (mg $100g^{-1}$ of fruit dry weight) of the major individual polyphenols in Radiance and Sensation strawberry cultivars at harvest (initial) and after 3 days at 1°C, 10°C and 20°C (Harvest 3).

Polyphenols	Radiance				Sensation				*p value*
	Initial	1°C	10°C	20°C	Initial	1°C	10°C	20°C	
Anthocyanidins									
Cyanidin	25.9±0.9 a [a]	19.5±0.9 abc	21.2±1.3 ab	16.5±3.6 abc	18.7±0.5 abc	9.6±0.4 c	11.4±0.8 bc	14.0±4.1 bc	0.0018
Pelargonidin	65.5±5.7 a	42.1±4.6 bc	34.2±1.6 bc	37.5±4.9 bc	49.0±4.0 ab	26.1±1.2 c	32.6±2.7 bc	27.2±2.8 c	<0.0001
Anthocyanins									
Cyanidin 3-glucoside	ND [b]	ND	0.1±0.1 b	2.8±1.8 a	ND	ND	ND	ND	0.0780
Pelargonidin 3-glucoside	408.5±41.5 ab	404.9±25.2 ab	462.0±50.3 a	412.4±139.4 ab	182.1±16.5 ab	83.9±6.0 b	130.0±27.6 ab	276.7±118.8 ab	0.0122
Flavonols									
Kaempferol 3-glucoside	102.6±9.5 a	98.4±13.6 a	47.4±5.0 bc	49.5±16.1 bc	78.1±0.7 ab	23.1±2.2 c	38.1±9.8 bc	47.9±2.4 bc	0.0001
Kaempferol	16.8±0.3 a	11.4±0.6 bc	12.1±1.0 bc	10.0±1.7 c	14.8±0.3 ab	8.3±0.3 c	9.5±0.8 c	8.6±0.8 c	<0.0001
Quercetin 3-glucoside	73.7±5.3 ab	55.4±6.3 bc	61.0±6.0 abc	48.6±7.1 bcd	86.8±0.7 a	23.4±1.3 d	37.9±12.0 cd	51.3±2.9 bc	<0.0001
Quercetin	31.4±4.7 a	3.8±2.2 b	22.4±2.5	6.7±1.7 a	1.2±0.4 b	0.5±0.5 b	3.4±1.7 b	4.1±0.5 b	<0.0001
Myricetin	33.8±1.8 ab	40.7±9.9 a	35.1±10.0 ab	23.5±6.6 ab	24.4±0.9 ab	10.7±0.4 b	11.87±1.1 ab	19.0±7.2 ab	0.0215
Flavanols									
Catechin	18.7±0.0 ab	11.9±0.3 b	10.6±0.4 b	14.3±7.1 b	14.7±0.0 b	20.0±4.0 ab	37.8±7.5 a	27.3±5.6 ab	0.0088
Epicatechin	676.1±72.5 a	667.1±57.0 a	751.9±90.9 a	900.6±88.2 a	295.9±42.0 b	127.6±15.2 b	198.7±54.7 b	257.7±19.3 b	<0.0001
Phenolic acids									
Caffeic Acid	26.5±8.5 b	40.9±5.8 a	23.3±4.7 b	2.2±1.0 c	ND	ND	ND	ND	<0.0001
p-Coumaric Acid	168.2±23.2 a	74.1±7.0 bc	79.3±10.3 b	60.0±25.0 bc	55.9±6.7 bc	9.7±1.4 c	17.7±5.8 bc	21.0±1.1 bc	<0.0001
Ferulic Acid	49.3±7.5 a	31.8±3.0 abc	36.6±4.7 ab	26.2±7.7 abc	39.6±3.9 a	9.8±1.0 c	14.0±3.2 bc	24.6±8.0 abc	0.0014
Gallic Acid	122.3±3.9 a	74.2±5.5 bc	55.4±5.4 cd	45.0±12.2 cd	95.0±3.4 ab	54.1±6.7 cd	51.6±3.3 cd	34.8±7.0 d	<0.0001
Chlorogenic Acid	121.6±13.7 a	156.4±15.7 a	136.3±14.0 a	134.4±12.5 a	41.4±8.7 b	19.0±1.6 b	9.7±4.6 b	1.8±0.6 b	<0.0001
Hydrolyzable tannins									
Ellagic Acid	19.0±0.7 a	12.9±0.6 abc	11.8±0.4 bc	8.4±1.6 c	16.2±1.0 ab	11.3±1.5 bc	9.5±1.5 c	7.2±1.6 c	0.0002

[a] Letters after averages denote significant differences ($p < 0.05$) between treatments based on Tukey's HSD test; averages followed by the same letter are not significantly different.
[b] ND = non-detected.

In our study, Radiance harvested at the beginning of the strawberry season had a greater concentration for all seventeen polyphenols after storage at 10 °C than at 1 °C (Table 3). In contrast, Sensation strawberries stored for 3 days at 10 °C had lower levels of most polyphenols than fruit stored at 1 °C (Table 3). Strawberry Radiance held at 20 °C showed higher or similar concentrations of polyphenols than fruit stored at 1°C, excluding kaempferol and gallic acid, which had lower concentrations in fruit stored at 20 °C (Table 3). In contrast, Sensation strawberries stored at 20 °C showed lower levels of most polyphenols than fruit stored at 1 °C, except for pelargonidin 3-glucoside, p-coumaric acid, ferulic acid, and chlorogenic acid (Table 3).

For the third harvest, Radiance strawberries stored at 1 °C had a greater concentration of most polyphenols compared to fruit stored at 10 °C, except for cyanidin, cyanidin 3-glucoside, kaempferol, quercetin 3-glucoside, quercetin, epicatechin, and ferulic acid, which were higher in samples stored at 10 °C (Table 4). In contrast, Sensation strawberries held at 10 °C had higher polyphenol levels than fruit stored at 1 °C, except for chlorogenic acid, which levels were higher in fruit stored at 1 °C. Strawberry Radiance stored at 20 °C showed higher levels of most polyphenols than fruit held at 1 °C, except for gallic acid, chlorogenic acid, and ellagic acid, which were higher in fruit kept at 1 °C.

Polyphenol Classes

Figures 3 to 8 show the levels of the major classes of polyphenols (i.e., anthocyanidins, anthocyanins, flavonols, flavanols, phenolic acids, and hydrolyzable tannins) in strawberries Radiance and Sensation, and the impact of the different storage temperatures on each group.

At harvest (initial), Radiance had higher levels of anthocyanidins (cyanidin and pelargonidin) than Sensation (Fig. 19). The levels of anthocyanidins tended to decline after storage, regardless of the cultivar or storage temperature. Also, the concentration of anthocyanidins tended to decrease as temperature increased, but differences between temperatures were not significant. Thus, it seems that storage temperature did not significantly impact anthocyanidins' levels in both strawberry cultivars.

The levels of anthocyanins (cyanidin 3-glucoside and pelargonidin 3-glucoside) were also higher in Radiance than in Sensation, regardless of the time of harvest and storage temperature (Fig. 20). However, the levels of anthocyanins were significantly higher in the third harvest (Fig. 20B) compared to the first

harvest (Fig. 20A). Although not statistically significant, there was an overall increase in the levels of anthocyanins as the temperature increased, particularly in the first harvest (Fig. 20A).

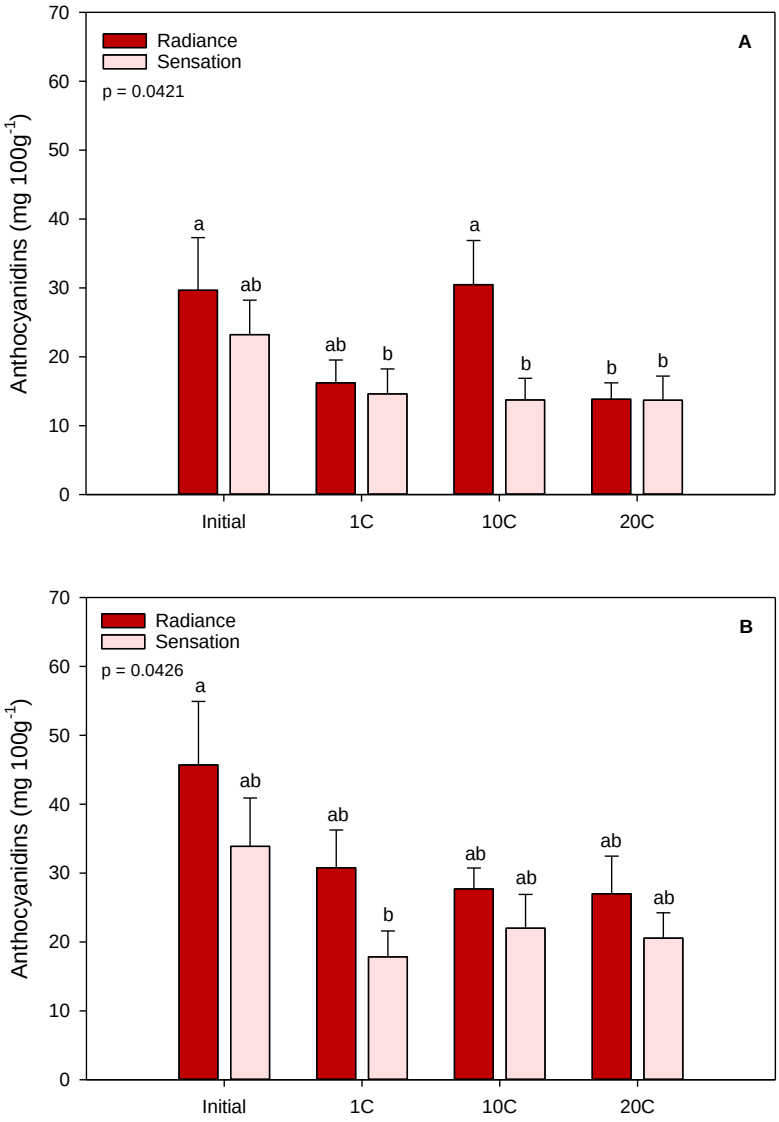

Figure 19. Anthocyanidin Content from HPLC Analysis for Radiance and Sensation. Anthocyanidins content (cyanidin and pelargonidin) for strawberries Radiance and Sensation at harvest (initial) and after 3 days of storage at 1°C, 10°C and 20°C. A = first harvest in January; B = third harvest in March. Bars are means of 3 biological replicates of 6 or 15 strawberries each, for the first and third harvests, respectively. Letters above each bar denote significant differences ($p < 0.05$) between treatments based on Tukey's HSD test; bars with the same letter are not significantly different.

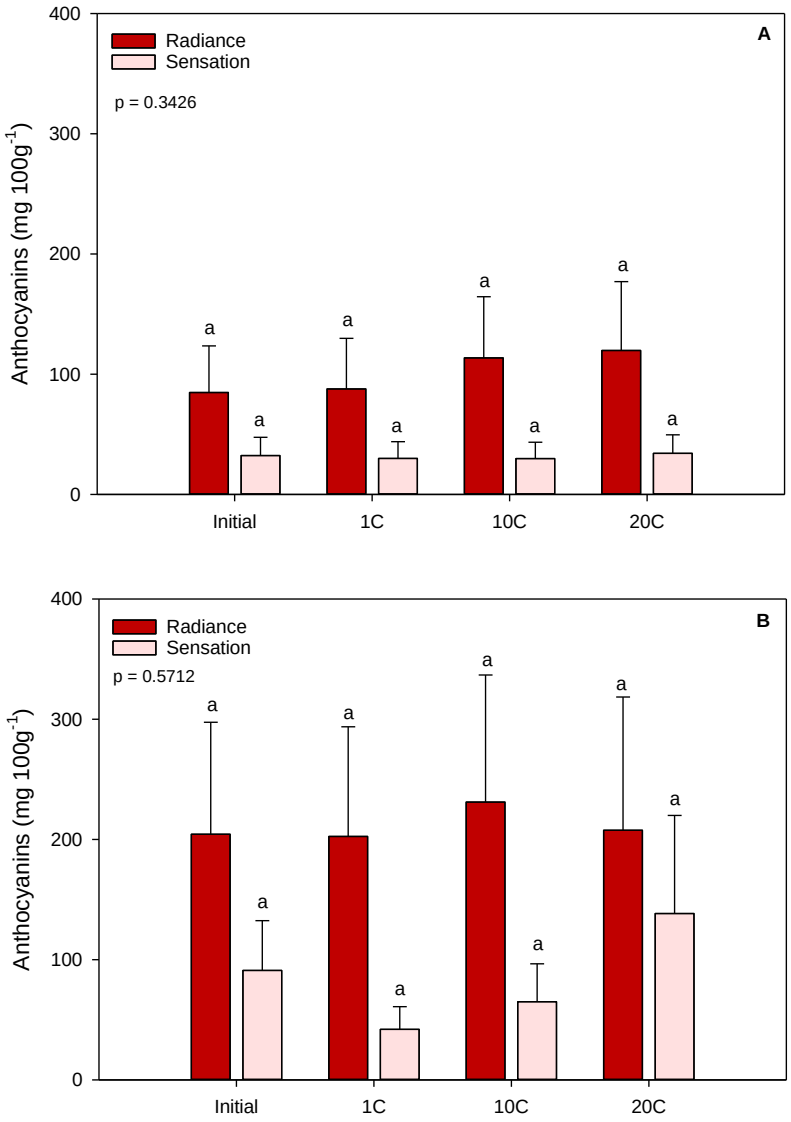

Figure 20. Anthocyanin Content from HPLC Analysis for Radiance and Sensation. Anthocyanin content (cyanidin 3-glucoside and pelargonidin 3-glucoside) for strawberries Radiance and Sensation at harvest (initial) and after 3 days of storage at 1°C, 10°C and 20°C. A = first harvest in January; B = third harvest in March. Bars are means of 3 biological replicates of 6 or 15 strawberries each, for the first and third harvests, respectively. Letters above each bar denote significant differences ($p < 0.05$) between treatments based on Tukey's HSD test; bars with the same letter are not significantly different.

The flavonol group is a large group of polyphenols comprised by kaempferol 3-glucoside, kaempferol, quercetin 3-glucoside, quercetin and myricetin. On average, strawberry Radiance had higher levels of flavonols than Sensation at the time of harvest and after 3 days of storage, regardless of the temperature (Fig. 21). The levels of flavonols in Radiance decreased after storage in the first harvest, particularly in fruit stored at 1°C (Fig. 21A). In Sensation, there was a marked decrease in the levels of flavonols as

temperature increased. In the third harvest, the trend was similar, but Sensation showed higher levels of flavonols after storage at 10 or 20 °C compared to fruit stored at 1 °C (Fig. 21B).

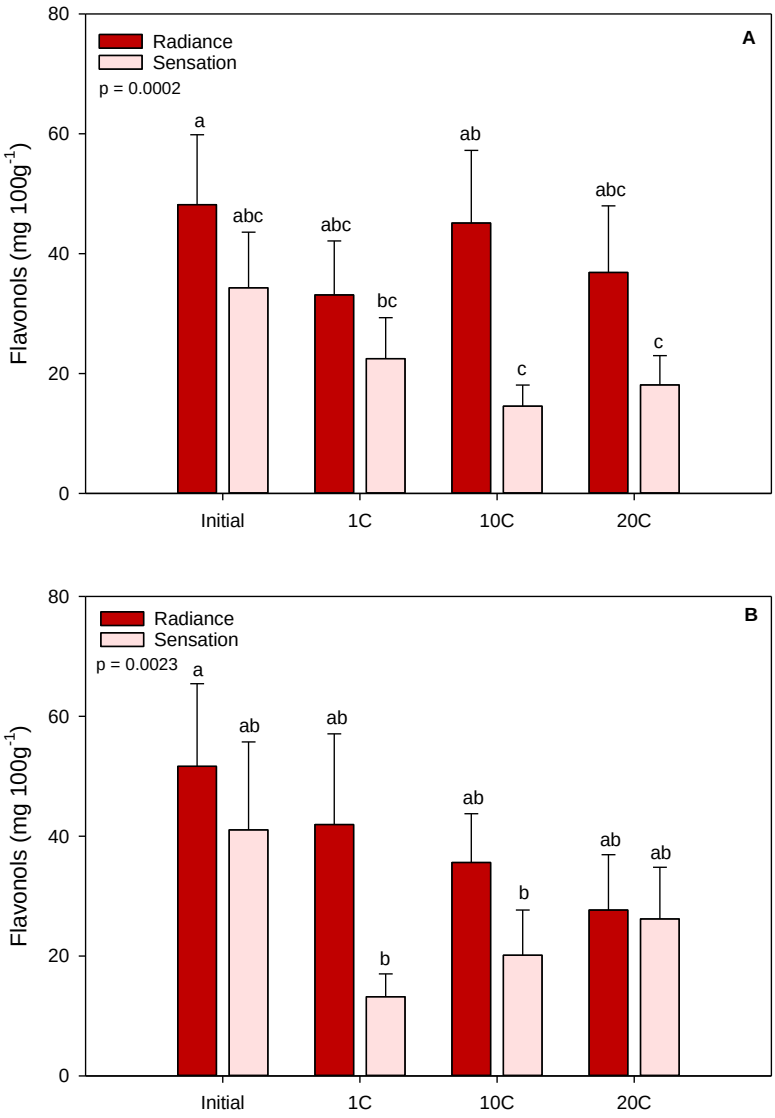

Figure 21. Flavonol Content from HPLC Analysis for Radiance and Sensation. Flavonol content (kaempferol 3-glucoside, kaempferol, quercetin 3-glucoside, quercetin and myricetin) for strawberries Radiance and Sensation at harvest (initial) and after 3 days of storage at 1°C, 10°C and 20°C. A = first harvest in January; B = third harvest in March. Bars are means of 3 biological replicates of 6 or 15 strawberries each, for the first and third harvests, respectively. Letters above each bar denote significant differences ($p < 0.05$) between treatments based on Tukey's HSD test; bars with the same letter are not significantly different.

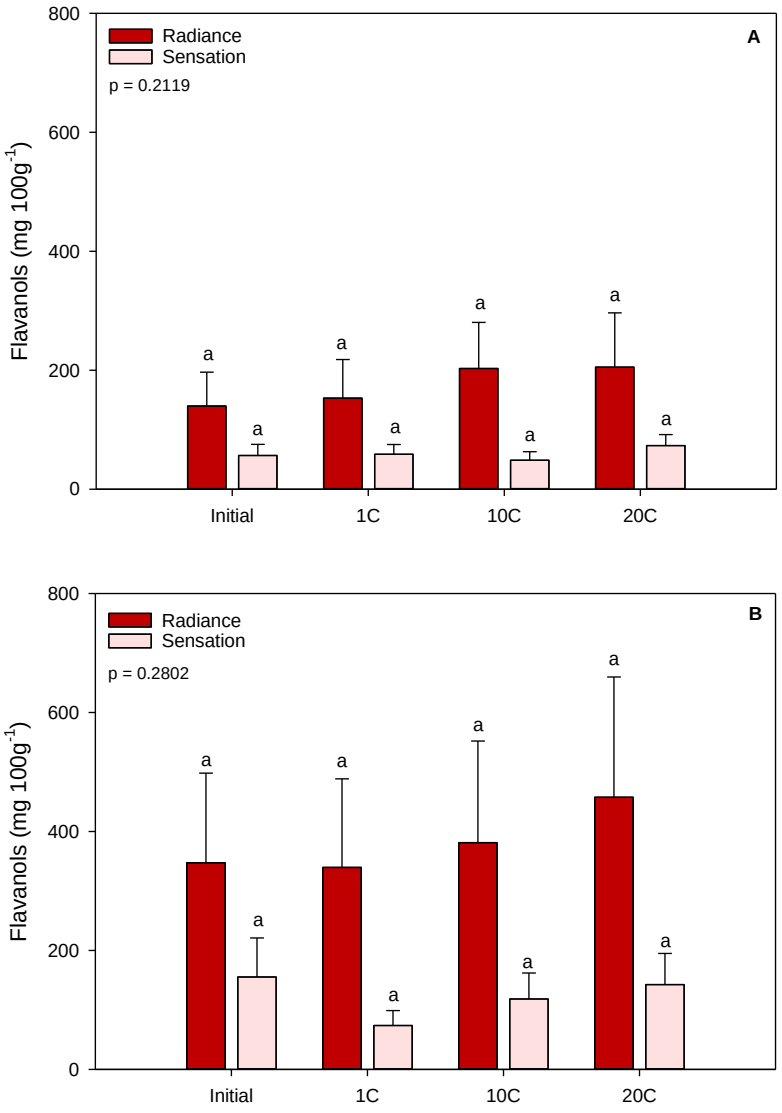

Figure 22. Flavanol Content from HPLC Analysis for Radiance and Sensation. Flavanols content (catechin and epicatechin) for strawberries Radiance and Sensation at harvest (initial) and after 3 days of storage at 1°C, 10°C and 20°C. A = first harvest in January; B = third harvest in March. Bars are means of 3 biological replicates of 6 or 15 strawberries each, for the first and third harvests, respectively. Letters above each bar denote significant differences ($p < 0.05$) between treatments based on Tukey's HSD test; bars with the same letter are not significantly different.

Radiance strawberries had significantly higher flavanols catechin and epicatechin levels than strawberry Sensation, regardless of the harvest (Fig. 22). However, the concentrations were substantially lower in the first harvest (Fig. 22A) than in the third harvest (Fig. 22B). Although not statistically significant, there was an apparent increase in the flavanol levels with increasing storage temperature, particularly for Radiance strawberries. Fruit from the third harvest stored at 20 °C had on average higher flavanol levels than fruit held at 1 or 10 °C (Fig. 22B).

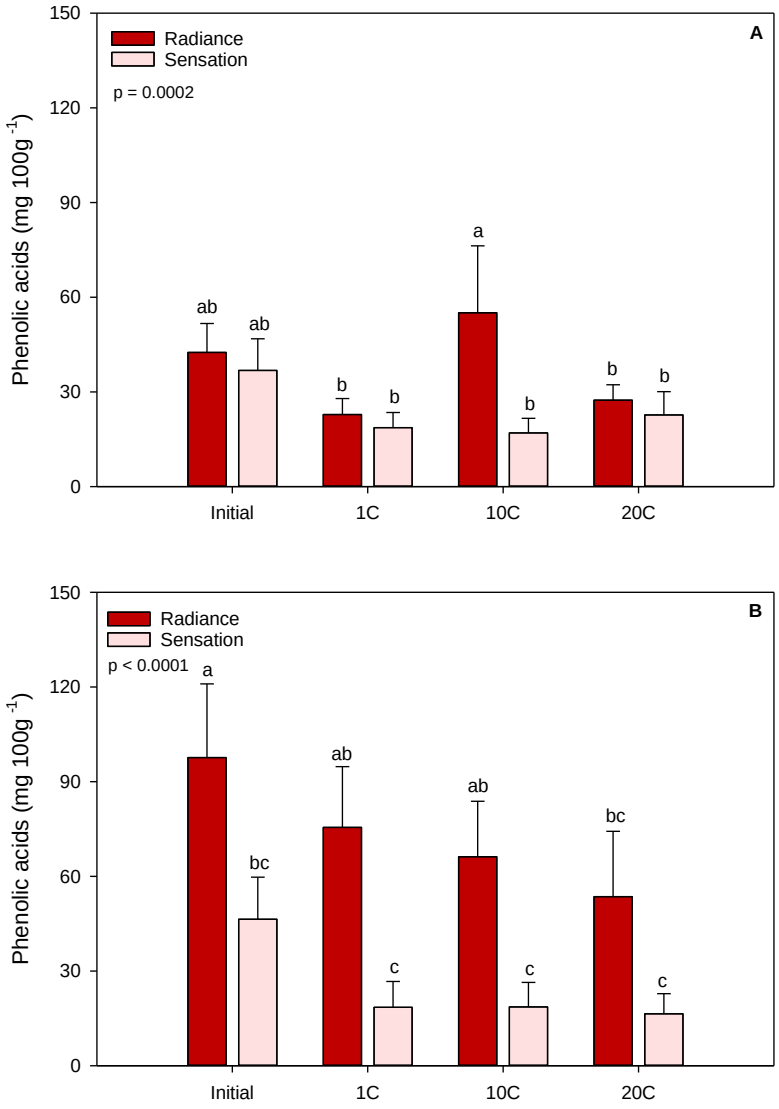

Figure 23. Phenolic Acid Content from HPLC Analysis for Radiance and Sensation. Phenolic acids content (caffeic, *p*-coumaric, ferulic, gallic and chlorogenic acids) for strawberries Radiance and Sensation at harvest (initial) and after 3 days of storage at 1°C, 10°C and 20°C. A = first harvest in January; B = third harvest in March. Bars are means of 3 biological replicates of 6 or 15 strawberries each, for the first and third harvests, respectively. Letters above each bar denote significant differences ($p < 0.05$) between treatments based on Tukey's HSD test; bars with the same letter are not significantly different.

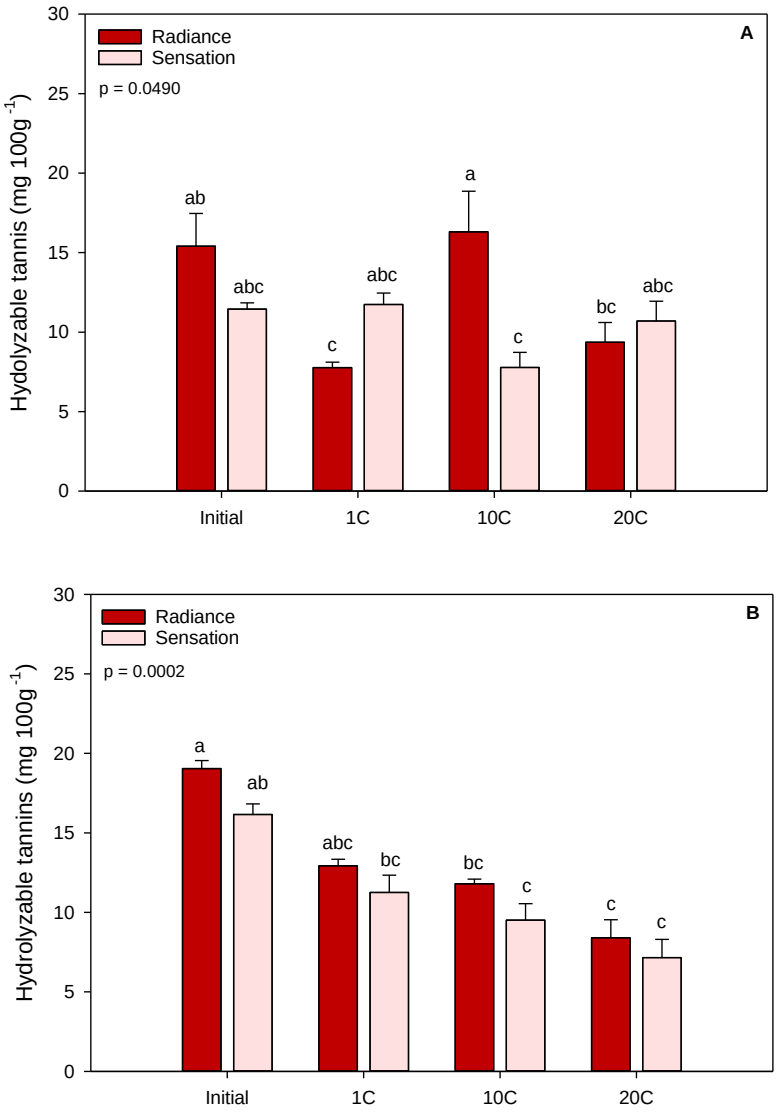

Figure 24. Hydrolyzable Tannin Content from HPLC Analysis for Radiance and Sensation. Hydrolyzable tannins content (ellagic acid) for strawberries Radiance and Sensation at harvest (initial) and after 3 days of storage at 1°C, 10°C and 20°C. A = first harvest in January; B = third harvest in March. Bars are means of 3 biological replicates of 6 or 15 strawberries each, for the first and third harvests, respectively. Letters above each bar denote significant differences ($p < 0.05$) between treatments based on Tukey's HSD test; bars with the same letter are not significantly different.

In Radiance, phenolic acids (caffeic, p-coumaric, ferulic, gallic, and chlorogenic acids) and hydrolyzable tannins (ellagic acid) were, in general, higher at harvest and after storage than in Sensation. However, levels tended to decrease as temperature increased (Figs. 23 and 24). This trend was evident in fruit from the second harvest where strawberries stored for 3 days at 20 °C had significantly lower phenolic acid and hydrolyzable tannin contents than those held at 1 or 10 °C (Fig. 23B and 24B).

Overall, the large standard errors (i.e., large sample variability) might have contributed to the lack of significance between treatments regarding some polyphenol groups. However, there was a clear difference between Radiance and Sensation concerning the levels of polyphenols, with Radiance showing, at harvest, consistently higher levels of all polyphenol groups than Sensation. There was also a significant harvest variation, most likely due to environmental conditions. Some studies have shown a significant difference in anthocyanin and total phenolic contents between different strawberry cultivars and variability in response to UV light exposure in terms of polyphenol accumulation (Cervantes et al., 2019; Palmieri et al., 2017). There was a more evident trend in the data from the third harvest where it was apparent that levels of anthocyanins, flavanols, and phenolic acids were, in particular, significantly higher than in the first harvest. Therefore, rainfall during the first harvest might have contributed to additional stress on the fruit, causing a more considerable variability and less homogeneous trend between temperature treatments. As discussed previously in Chapter 3, excessive rain can significantly impact the quality of the strawberry fruit (Herrington et al., 2011). Rain can cause cellular breakdown and stress to the fruit (Herrington et al., 2011). However, the effect of storage temperature on strawberry Radiance and Sensation polyphenol profiles cannot be generalized. While it seems that there was a tendency to decline the levels of anthocyanidins, flavonols, phenolic acids, and hydrolyzable tannins with increasing the storage temperature, anthocyanins and flavanols tended to increase as storage temperature increased.

On average, regardless of the harvest, strawberry Radiance had higher total polyphenols (sum of all polyphenols) at harvest and after storage than Sensation (Fig. 25). However, strawberries from the first harvest (Fig. 25A) had significantly lower total polyphenol levels than fruit from the third harvest (Fig. 25B). Adhikary et al. (2020) showed that in pears, a higher respiration rate (RR) correlates with an increase in polyphenol oxidase (PPO) activity (Adhikary et al., 2021). While this study was not done on strawberries, it likely explains the significant difference in polyphenolic content between harvests found in our research. That is, strawberries Radiance and Sensation from the first harvest had significantly higher RR than fruit the third harvest (Chapter 3) and possibly higher PPO activity (not measured) which may have led to lower total polyphenol levels.

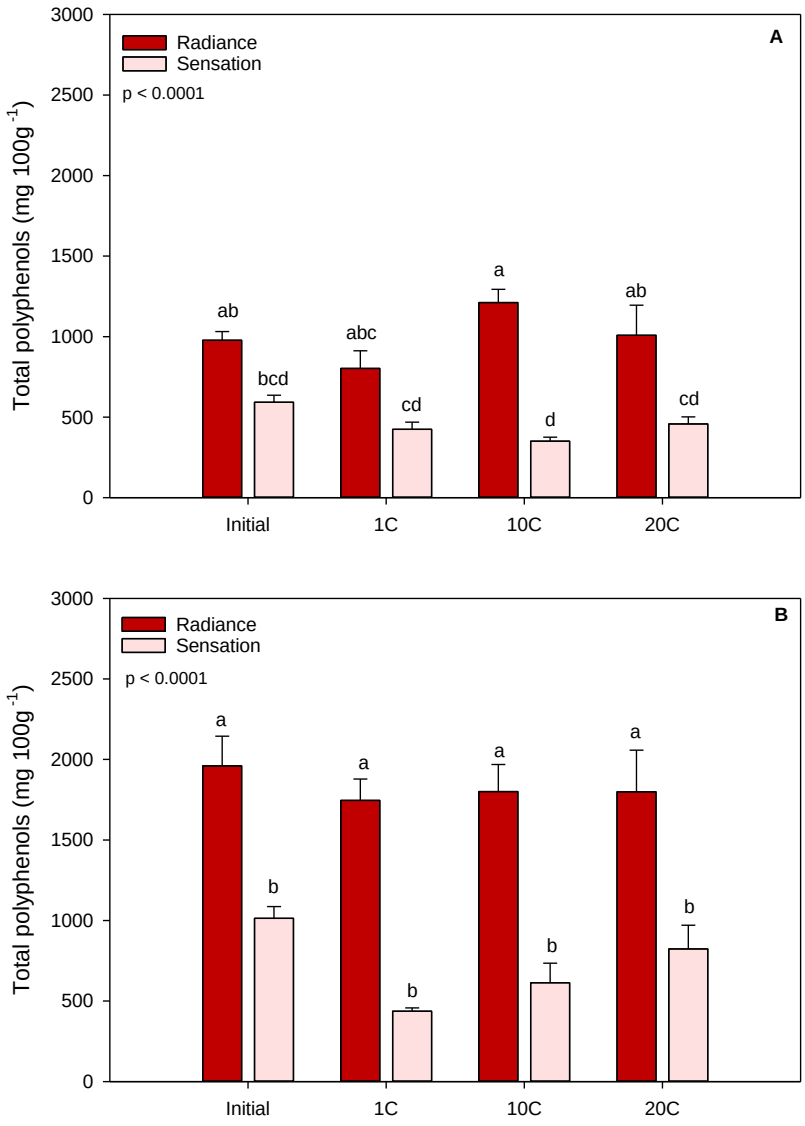

Figure 25. Total Phenolic Content from HPLC Analysis for Radiance and Sensation. Total polyphenolic content (i.e., sum of all polyphenols detected) for strawberries Radiance and Sensation at harvest (initial) and after 3 days of storage at 1°C, 10°C, and 20°C. A = first harvest in January and B = third harvest in March. Bars are means of 3 biological replicates of 6 or 15 strawberries each, for the first and third harvests, respectively. Letters above each bar denote significant differences ($p < 0.05$) between treatments based on Tukey's HSD test; bars with the same letter are not significantly different.

In the first harvest, Radiance strawberries stored at 10 °C had a greater increase in concentration from initial values than the samples stored at 20 °C, whereas there was a slight decrease in strawberries held at 1°C (Fig. 25A). However, there was a slight but non-significant decline in Radiance's total polyphenol levels after storage at different temperatures in the third harvest. In contrast, Sensation stored at 10 and 20 °C had higher levels than fruit held at 1°C (Fig. 25B). Storage at 10°C has been shown to contribute to an

increase in the anthocyanin content of strawberries on a fresh-weight basis (Ayala-Zavala et al., 2004; Shin et al., 2007). However, others have also shown that strawberries stored at 20 °C had a greater concentration of total phenolic and anthocyanin contents on a fresh-weight basis than the fruit held at 10 °C. In our study, Radiance strawberries from the first harvest seem to be an outlier as fruit stored at 10 °C had higher levels of total polyphenols than fruit kept at 20 °C (Fig. 25A). For Sensation, the fruit stored at 20 °C had the greatest concentration of anthocyanins, consistent with the literature (Ayala-Zavala et al., 2004; Shin et al., 2007). For both cultivars, there was no significant difference between the initial values at harvest and those after storage at 1°C. It was shown by Li et al. (2021) that during storage at 20°C the genes responsible for the regulation of polyphenols, and particularly, anthocyanin biosynthesis had an increase in expression over a 3-day storage period (Li et al., 2021). Thus, *de novo* synthesis during stress temperature storage possibly explains the increase in anthocyanin content in Sensation (Fig. 20B). However, further studies are needed to support this hypothesis.

Khattab et al. (2015) looked at the effect of long-term frozen storage on the total phenolic and anthocyanin contents of whole haskap berries (*Lonicera caerulea* L.). While the haskap berries are not strawberries, they share several major polyphenols (Caprioli et al., 2016; Giampieri et al., 2012). Thus, Khattab et al. (2015) showed a steady decline in anthocyanin content (using cyanidin-3-glucoside as the anthocyanin standard) during a six-month freezing storage period at -18 °C. In our study, strawberry samples were kept frozen at -30°C for more than two years, and thus there could have been an unaccounted-for loss of certain polyphenols during freezing. A previous study also suggested that freezing storage for 7 to 9 months at -20 °C might influence strawberries' phenolic contents (Häkkinen et al., 2000). Furthermore, it seems that kaempferol and myricetin are more susceptible than quercetin to degradation during freezing. In strawberries, quercetin content increased by 32%, whereas kaempferol could not be quantified after 9 months of storage at 20 °C (Häkkinen et al., 2000).

Conclusions

Radiance and Sensation strawberry cultivars showed significantly different polyphenol profiles at harvest and after storage. Overall, Radiance had a greater concentration of individual and total polyphenols than Sensation. These results agree with the data from Chapter 3, with Radiance having a

greater concentration of total phenolic and anthocyanin contents compared to Sensation. Temperature stress seems to have a different effect on each polyphenol group because there was a tendency to decline with increased temperature for some polyphenol groups such as anthocyanidins, flavonols, phenolic acids, and hydrolyzable tannins, anthocyanin. At the same time, the levels of flavanols tended to increase as storage temperature increased. Further studies should investigate the impact of pre and postharvest stress on the polyphenol profiles of other strawberry cultivars. Also, the effect of freezing duration on polyphenol changes and degradation of individual polyphenols during long-term freezing storage should be investigated.

CHAPTER FIVE:

CHARACTERIZING AND COMPARING THE POLYPHENOLIC PROFILES OF THE WHITE-FRUITED STRAWBERRY 'FLORIDA PEARL' AGAINST THE STANDARD, RED-FRUITED STRAWBERRY 'FLORIDA BRILLIANCE'

Introduction

The white Chilean strawberry *Fragaria chiloensis* spp*. chiloensis* f. chiloensis is a native of southern Chile and one of the progenitors of the commercial strawberry (*Fragaria* × ananassa Duch.) White strawberries were initially called "Pineberries" and were very appreciated in Europe in the 19th century. In Chile, they are still prevalent, becoming trendier in other parts of the world (Finn et al., 2013; Ulrich & Olbricht, 2016). In Europe, the white strawberry cultivar 'Snow White', for example, has become a gourmet fruit in recent years (Ulrich & Olbricht, 2016). In Florida, the first white-fruit strawberry, 'Florida Pearl', appeared on the market around 2018, but so far, no data have been published on its biochemical characteristic and postharvest performance.

Anthocyanins are the class of polyphenolic compounds responsible for the red color of the strawberry fruit, also being significant contributors to the total phenolic content of the fruit (Aaby et al., 2012). However, earlier studies by Simirgiotis et al. (2009) showed that total anthocyanin and ellagic contents were about 13 and 11 times higher in 'Chandler' strawberries than in the white form of *F. chiloensis*. White strawberries varieties lack the typical red coloring and have significantly lesser anthocyanins (Salvatierra et al., 2010. Several enzymes in the biosynthetic anthocyanin pathway are downregulated in white strawberry cultivars (Hjerno et al., 2006). The down-regulated enzymes are chalcone synthase, dihydroflavonol reductase, flavanone 3-hydroxylase, and methyltransferase. These enzymes belong to the flavonoid biosynthetic pathway, which produces several compounds, including pelargonidin, which gives the red color to strawberry fruit (Hjerno et al., 2006). Unlike in the red-fruited strawberry varieties, cyanidin 3-glucoside is the major anthocyanin in white fruit, followed by pelargonidin 3-glucoside (Simirgiotis & Schmeda-Hirschmann, 2010; Simirgiotis et al., 2009).

Like almost all living organisms, strawberry fruit also has an internal mechanism to protect itself from UV radiation. When anthocyanins are synthesized, the development of the red pigmentation occurs, protecting the fruit from UV ration (Smillie & Hetherington, 1999; Steyn et al., 2002). This protection mechanism can be seen as a deepening of the strawberry fruit's red coloring and an increase in the synthesis of anthocyanins. Besides, UV rays and field temperatures can significantly impact the synthesis of other polyphenols on strawberry fruit. It has been previously shown that temperature stress such as high temperatures both pre and post-harvest can lead to an increase in the metabolism of the fruit resulting in a higher respiration rate. With an increased respiration rate, the strawberry fruit will be more likely to breakdown its primary (i.e., sugars and organic acids) and secondary metabolites (i.e., polyphenols) at a much faster rate (Wang & Camp, 2000)

The main objective of this study was to characterize and compare the polyphenolic profiles at harvest and during postharvest of the commercial standard, red-fruited strawberry 'Florida Brilliance' and first Florida white-fruited 'Florida Pearl' strawberry. To accomplish our objective, both strawberry cultivars were harvested three times during the 2021 strawberry season. Color, total phenolics, anthocyanin contents, and polyphenol profiles were evaluated at harvest and after cold storage. To our knowledge, this is the first study characterizing and comparing the polyphenol profiles of the white-fruited strawberry 'Florida Pearl' against the commercial red strawberry cultivar 'Florida Brilliance' at harvest and during postharvest cold storage.

Materials and Methods

Plant Material

The two strawberry cultivars used for this research were 'Florida Brilliance' and 'Florida Pearl' (hereafter referred to as Brilliance and Pearl, respectively). Both cultivars were harvested from fields at the University of Florida Gulf Coast Research and Education Center (GCREC) in Wimauma, Florida. Samples were harvested three times during the 2021 season (January 18 - first harvest, February 10 - second harvest, and March 1 - third harvest), transported within one hour of harvest to the University of South Florida Food Quality Laboratory in Tampa, and then sorted into clamshell replicates. Two flats of Brilliance and one flat of Pearl were collected for the first harvest, containing ~300 and ~120 individual fruits, respectively. For the

second and third harvests, two flats of Brilliance and two flats of Pearl having ~300 individual fruits were harvested. For the experimental set-up, 10 fruits per clamshell replicate (21 clamshells total; 3 replicates for each day and temperature) were used for Brilliance. For Pearl, 10 fruits per clamshell replicate were used for the second and third harvests; however, for the first harvest, due to pre-harvest frost and intense cold temperatures that caused a smaller yield, only 5 fruits were used per clamshell.

Storage Conditions

As prior studies have shown, 1 °C and high relative humidity (RH) are the optimum postharvest storage conditions for strawberries (Cayo et al., 2016; Kelly et al., 2019; Kelly et al., 2016). Therefore, strawberry samples were stored at 1 ± 0.2°C and 65.0 ± 0.5% RH (VPD = 0.23 KPa) for up to nine days inside Forma Environmental Chambers (Model 3940 series, Thermo Electron Corporation, Ohio, USA).

Weight Loss and Moisture Content

The weight loss of each strawberry cultivar was determined by weighing the samples at harvest and after nine days of storage. Clamshell replicates contained 10 individual fruits, except for Pearl in the first harvest, where the clamshells held 5 fruits per replicate. The moisture content of the samples was determined by drying three weighed aliquots of pureed strawberry tissue at 80°C for 24 hours until weight stabilized. Moisture content measurements were performed on the day of harvest. Weight loss and moisture content were necessary attributes to allow for data expression in dry weight, which takes water loss during storage into account. The following formula was used for water loss corrections: [chemical components (fresh weight) $\times$ 100 g / strawberry dry weight + weight loss during storage (g)].

Instrumental Color Analysis

The color analysis was performed as described previously by Kelly et al. (Kelly et al., 2016). A total of two measurements were taken on the opposite sides at the flattest area on the equatorial part of the fruit. A hand-held tristimulus reflectance colorimeter (Model CR-400, Minolta Co., Ltd., Osaka, Japan) equipped with a glass light-projection tube (CR-A33f, Minolta Co., Ltd., Osaka, Japan) was utilized. The color was recorded using the CIE-L*a*b* uniform color space (CIE-Lab), L* (lightness), a* (redness), and b* (yellowness) values. The numerical values of a* and b* readings were converted into hue angle using the Konica Minolta CR-400 Utility software CR-S4w (2002–2010 Konica Minolta Sensing, Inc., Osaka, Japan).

These readings were performed on the day of harvest and after 9 days of storage before homogenization of the fruit for further testing.

Total Phenolic Content

Total phenolic content was determined using the Folin-Ciocalteau (F-C) assay described by Nunes et al. (Nunes et al., 2005). This assay uses Folin-Ciocalteau's phenol reagent as an oxidizing agent to reduce tyrosine and tryptophan residues resulting in blue color with a maximum absorbance at 765 nm (Sánchez-Rangel et al., 2013). The phenolic compounds within the sample interact with the F-C reagent at a pH of ~10, which was achieved by adding a sodium carbonate solution. The absorbance level is proportional to the concentration of phenolic compounds within the sample (Sánchez-Rangel et al., 2013). To obtain juice, pureed fruits were centrifuged for 20 minutes at 4500 rpm using a refrigerated centrifuge (4ºC). The light-colored supernatant was filtered through cheesecloth into 50 ml centrifuge tubes. Then 0.5 g of supernatant was diluted in 9.5 ml of ultrapure deionized water. Each sample replicate was evaluated in triplicate to account for error. Five mL of diluted F-C reagent (1:9 reagent to water dilution) was added to each diluted sample and vortexed for 5 seconds. Between 30 seconds and 8 minutes after adding the F-C reagent, 4 ml of sodium carbonate (0.71 M) solution was added, and each sample was vortexed for 5 seconds. The samples were incubated at 30 ºC for 1 hour then chilled on ice for 30 minutes. The absorbance was read at 765 nm on a microplate reader and then plotted on a standard curve to determine the concentration of total soluble phenolic compounds. Gallic acid was used to create the four standards for this assay. The standard concentrations were comprised of 0.04 mg/ml, 0.08 mg/ml, 0.12 mg/ml, and 0.16 mg/ml. Total phenolic content was reported on a dry weight basis to compensate for water loss during storage.

Total Anthocyanin Content

The concentration of anthocyanin contents was determined using the method described by Nunes et al. (Nunes et al., 2005). Two grams of pureed fruit were mixed with 18 ml 0.5% (v/v) HCL in methanol in foil-covered glass jars. The pigments were extracted for 1 hour at 4 ºC in the dark then filtered through a double layer of tissue. The samples were then read at 520 nm absorbance to determine the total anthocyanin contents. The anthocyanin pigments are light-sensitive; therefore, the entire process was performed under low light conditions. Anthocyanin contents were reported on a dry weight basis to compensate for water loss during storage.

Polyphenol Extraction

The extraction of the polyphenols was performed as described previously by Abountiolas and Nunes (2018) with minor adjustments such as using the fruit's puree instead of juice. Triplicates of 5 g of homogenate were mixed with 15 mL of acetone and homogenized using a polytron for 1 minute, then sonication for 10 minutes, and finally filtered through Whatman paper No.4. The filtrate was concentrated to 5 mL in an SPD121P SpeedVac® Concentrator (Thermo Fisher Scientific Inc., Asheville, NC, USA) and finally passed through a classic C18 Sep-Pack cartridge (Waters Technologies Corp., USA). The Sep-Pack cartridge was activated with ~5 mL each methanol followed by ultrapure water and then 3% acidified water before passing the concentrated sample. Anthocyanins and other phenolic compounds present were absorbed into the cartridge, while sugars, acids, and other water-soluble compounds were eluted with ~10 mL acidified water. The phenolic compounds were then recovered by passing ~2 mL of methanol (containing 3% formic acid) through the cartridge. The extracted sample was filtered through a 0.20 µm syringe filter into 2 mL autosampler vials and stored at -30°C until the time of analysis.

Individual Polyphenol Analysis

Analysis of phenolic compounds was conducted using a Hitachi LaChroma Ultra HPLC system coupled with a photodiode array detector (Hitachi, Japan) as according to Kelly (2018) with minor adjustments regarding retention times and dry weight conversions. Samples were injected at 40°C onto a reverse-phase Hypersil Gold C18 column (100 × 2.1 mm; particle size, 1.9 µm) (Thermo Fisher Scientific Inc., USA). The two mobile phases consisted of acidified water containing 0.5% formic acid (mobile phase A) and 0.1% formic acid in acetonitrile (mobile phase B) in an isocratic mixture. The flow rate was 0.3 µL/min, the wavelength detection included 250, 280, 360, and 520 nm, and the sample injection volume was 10 µL. Retention times and spectra were compared with pure standards of 17 compounds (Table 1) (at concentrations of 0.005 mg/mL, 0.01 mg/mL, and 0.1 mg/mL) from different polyphenol classes: flavonoids (cyanidin, pelargonidin, cyanidin 3-glucoside, pelargonidin 3-glucoside, quercetin, kaempferol, quercetin 3-glucoside, kaempferol 3-glucoside, myricetin, catechin, and epicatechin), phenolic acids (p-coumaric acid, ferulic acid, caffeic acid, gallic acid, and chlorogenic acid) and hydrolyzable tannins (ellagic acid). The flavonoids can be further classified into anthocyanidins (cyanidin and pelargonidin), anthocyanins (cyanidin 3-glucoside, and pelargonidin 3-glucoside), flavonols (quercetin, kaempferol, quercetin 3-glucoside,

kaempferol 3-glucoside, and myricetin), and flavanols (catechin and epicatechin). Quantification of the individual polyphenols will be based on the concentration of mg/100g fruit in dry weight.

Statistical Analysis

The Statistical Analysis System computer package (SAS Institute, Inc., 2004) was used to analyze the data from these experiments. The data were analyzed via three-way ANOVA analysis with cultivar, storage temperature, and storage time as the independent variables. Statistical analysis showed a significant difference between harvests leading to separate analysis for each harvest. Significant differences between treatments were detected using Tukey's Studentized Range (HSD) test at the 5% significance level.

Results and Discussion

Weight Loss

As expected, weight loss increased during storage, regardless of the cultivar (Fig. 26). However, weight loss increased as the season progressed, with Brilliance and Pearl strawberry cultivars harvested in March having the highest weight loss (11.2% and 9.8%, respectively) than the fruit harvest in January (7.0% 7.1%, respectively) or February (9.1% and 7.4%, respectively). The higher field temperatures measured at harvest in March (Table 5) than in January and February could have contributed to a greater water loss during storage. Our previous results showed that a higher temperature during postharvest could lead to a higher respiration rate and increased water loss (see Chapter 3). Although the negative impact of abuse postharvest temperature on strawberry quality has been well documented, preharvest field temperatures also affect the quality of the fruit. For example, studies have reported the effect of preharvest temperatures on strawberry weight loss. Besides, Barrios et al. (2014) showed that the respiration rate of strawberries grown at higher temperatures (from 10 to 23C) significantly increases due to accelerated metabolic reactions (Barrios et al., 2014). As shown in Table 5, by March, a significant increase in the environmental temperature could have been a considerable factor contributing to the increased weight loss observed throughout the season and possibly greater respiration rate.

Table 5. Weather conditions at harvest in the 2021 strawberry season.[a]

	Temperature (°C)			Rainfall	Daily (Max AVG)
Harvest date	Max	Min	AVG	(mm)	UV Index
Jan 18	17.8	6.1	12.5	0	4
Feb 10	27.2	18.9	21.1	0	5
Mar 1	28.3	21.7	24.3	0	6

Max = maximum; Min = minimum; AVG = average.
[a] Source: Florida Automated Weather Network (https://fawn.ifas.ufl.edu/data/reports).

Strawberry Pearl had significantly lower weight loss after 9 days of storage than Brilliance except in the first harvest, where there was not a significant difference between weight losses of both cultivars. As stated previously in Chapter 3, the differences in morphological characteristics between genotypes (e.g., the thickness of the cuticle and achene and stomata density) may also have contributed to differences in weight loss between cultivars (Cayo et al., 2016).

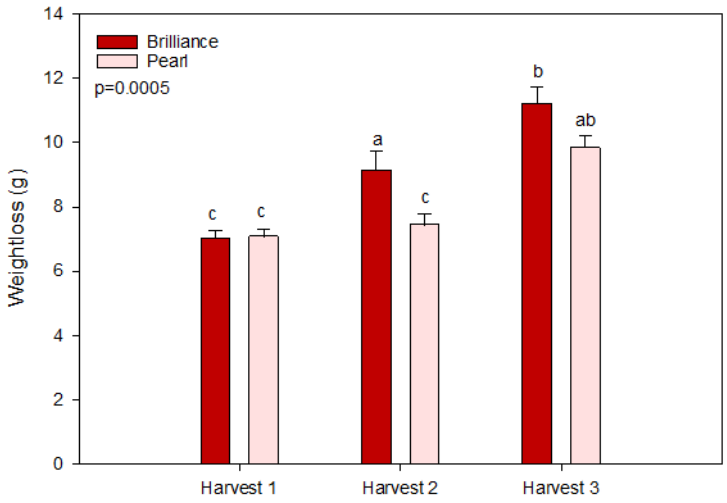

Figure 26. Weight Loss for Brilliance and Pearl. The average weight loss for Brilliance and Pearl strawberries after 9 days in storage at 1°C. Letters above each bar denote significant differences ($p < 0.05$) between treatments based on Tukey's HSD test; bars with the same letter are not significantly different.

Color

There was a visible change in Pearl strawberry's overall appearance and surface color not only after storage but also from harvest to harvest (Figs. 27 and 28). In January, the fruit appeared much lighter red at harvest than the fruit harvested later in the season, which had a noticeable red blush. After storage, Pearl strawberries lost their creamy appearance developing a yellowish discoloration. Brilliance also had a visible

color change, from a brighter light red to a darker red as the season progressed (Figs. 27 and 28). After storage, the red coloration increased, and the fruit appeared redder and less glossy than at harvest.

Data from instrumental color analysis confirmed the subjective appearance observations of both strawberry cultivars (Fig. 29). Overall, at harvest and after storage, Pearl had significantly higher L* (lighter) and hue angle (less red) values than Brilliance. These results were expected as Brilliance is a very red strawberry. For the a* value, Brilliance had significantly higher values (redder) than Pearl for all three harvests dates both at harvest and after storage. As discussed in the previous chapters, anthocyanins are the primary pigment contributing to the red color of strawberries. It has also been found that exposure to UV rays can increase anthocyanin synthesis (Anttonen et al., 2006; Cominelli et al., 2008). For the February (second) and March (third) harvests, not only was the average temperature higher than the January (first) harvest, but also the average UV index was also greater (Table 5). The increase in UV index as the season progressed, along with the stress of the field heat, likely contributed to an increase in anthocyanin synthesis and enhancement of red coloring on strawberry Pearl.

Figure 27. Strawberry cultivars Florida Brilliance and Florida Pearl on the day of harvest. The appearance of strawberry cultivars Florida Brilliance (above) and Florida Pearl (below) on the day of harvest (Day 0; Initial). A) January harvest; B) February harvest; C) March harvest.

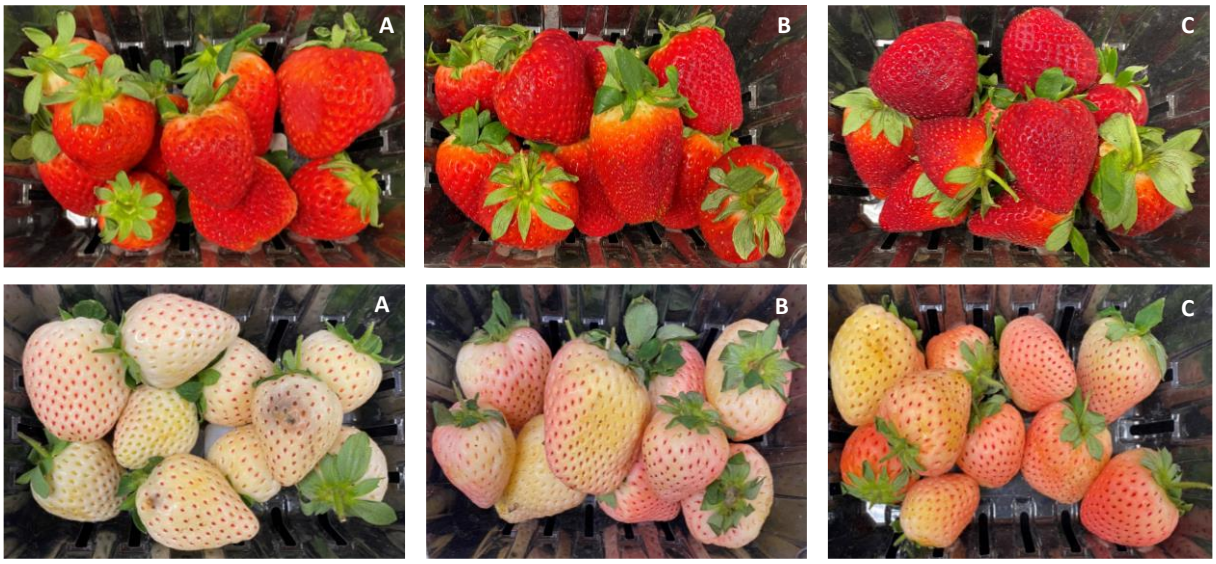

Figure 28. Strawberry cultivars Florida Brilliance and Florida Pearl after 9 days of storage at 1°C. The appearance of strawberry cultivars Florida Brilliance (above) and Florida Pearl (below) after 9 days of storage at 1°C. A) January harvest; B) February harvest; C) March harvest.

Anthocyanins have been previously suggested to play a major role in UV protection within the strawberry fruit (Smillie & Hetherington, 1999; Steyn et al., 2002). Since Pearl strawberry lacked a significant amount of these protective biochemical compounds, as the UV index increased, the production of anthocyanins needed to increase to allow the fruit to grow fully. The effect of UV radiation on the fruit increased the redness of strawberry Pearl. It was also confirmed by the significant increase in total anthocyanin and total phenolic contents over the season (Fig. 30).

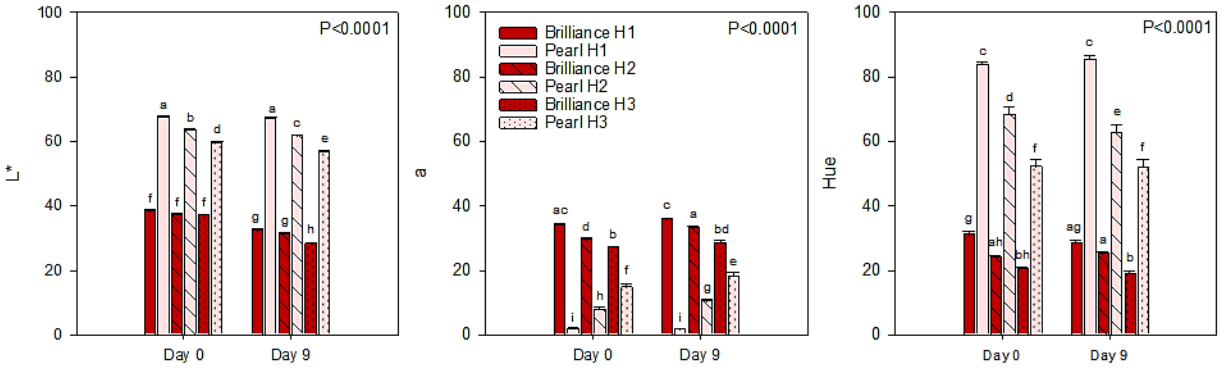

Figure 29. L*, a*, and Hue of Brilliance and Pearl. L* value ((brightness), a* value (redness), and Hue of strawberries Brilliance and Pearl on day of harvest (day 0) and after 9 days of postharvest storage at 1° C for three harvests (January, February, and March) during the 2021 season. Letters above each bar denote significant differences ($p < 0.05$) between treatments based on Tukey's HSD test; bars with the same letter are not significantly different.

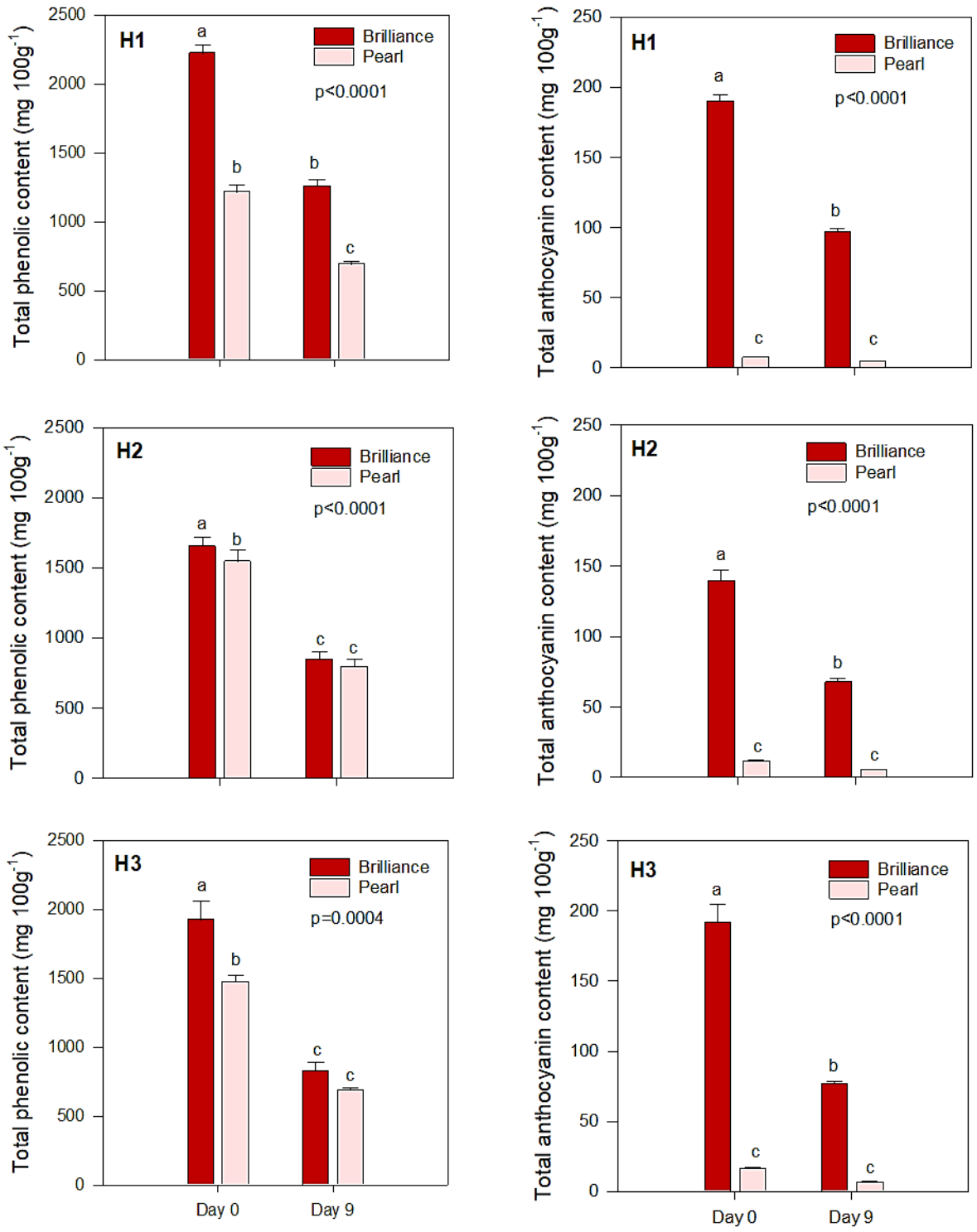

Figure 30. Total Anthocyanin and Total Phenolic Contents of Brilliance and Pearl. Total anthocyanin and phenolic contents of strawberries Brilliance and Pearl at the day of harvest (Day 0) and after 9 days of storage at 1°C. H1) First harvest in January; H2) Second harvest in February; H3) Third harvest in March. Letters above each bar denote significant differences ($p < 0.05$) between treatments based on Tukey's HSD test; bars with the same letter are not significantly different.

Total Phenolic and Total Anthocyanin Contents

Overall, total phenolic content (TPC) was slightly higher in Brilliance than in Pearl, regardless of harvest time (Fig. 30). Comparatively to the TPC measured in Pearl strawberries (1.41 g $100g^{-1}$ dry weight), previous studies have reported similar values for white strawberries (2.72 g $100g^{-1}$ dry weight) (Simirgiotis & Schmeda-Hirschmann, 2010). However, during storage, the TPC significantly decreased for both cultivars. Both Brilliance and Pearl showed a 43% decrease in TPC after 9 days of storage for the first harvest. Both cultivars showed a 49% decrease after storge for the second harvest. Finally, Brilliance had a 57% decrease for the third harvest, whereas Pearl had a 53% decrease after 9 days of storage. While Brilliance had a more significant weight loss than Pearl (Fig. 26), Pearl has a genetic difference in the types and amounts of polyphenols present within the fruit, which could be a factor in the similar declines in TPC.

Strawberry Brilliance had, on average, a significantly greater concentration of total anthocyanin contents on the day of harvest and after 9 days of storage (173.7 and 80.2 mg 100g-1 dry weight, respectively) compared to Pearl (11.6 and 5.5 g mg^{-1} dry weight, respectively) (Fig. 30). Similarly, Simirgiotis et al. (2009) also reported that the total anthocyanin content in ‘Chandler’ strawberries was 13 higher than in white-fruited cultivars. The levels of anthocyanins measured at harvest in Pearl (11.5 mg $100g^{-1}$ on average) was, however, much lower compared to data previously reported (43.6 mg $100g^{-1}$ dry weight) by Simirgiotis et al. (2010), possibly due to cultivar and environmental differences. As the season progressed, there was an increase in the initial (at harvest; day 0) total anthocyanin content in Brilliance and Pearl strawberries which agrees with the data obtained from analytical color measurements and visual observations (Figs. 2-4). These findings were most likely due to the increased UV radiation the fruits were exposed to during growth and maturation (Table 5). Although there was an increase throughout the season, there was still a consistent and significant decrease in anthocyanin content after storage for both cultivars (Fig. 30). Brilliance showed a 49% decrease in ANC for the first harvest, while Pearl had a 41% decrease. Brilliance and Pearl strawberries were much closer in terms of percent decrease from initial values at harvest to after storage for the second and third harvests. Brilliance ANC decreased by 52 and 60%, respectively, and Pearl saw a reduction of 53 58%, respectively. For two out of the three harvests, Brilliance had a more significant decrease in total anthocyanin content than Pearl. Brilliance also had a significantly more significant amount of anthocyanins than Pearl, so a greater decline was expected. Pearl most likely

has a downregulated synthesis of anthocyanins to maintain its whitish or creamy color. As with the Chilean white strawberry *F. chiloensis* (Salvatierra et al., 2010; Salvatierra et al., 2013; Saud et al., 2009), it was also expected that Pearl would have significantly lower amounts of anthocyanins compared to the red-fruited cultivar.

Overall, Brilliance had a more significant weight loss compared to Pearl. It has been discussed previously how increased weight loss (i.e., moisture loss) is associated with a more substantial loss of total soluble polyphenols (Nunes et al., 2005). Thus, for Brilliance, the breakdown of the anthocyanins at such a higher rate during postharvest could eventually explain why this red-fruited cultivar had a more significant decrease in total phenolic content after 9 days of cold storage even though both cultivars had a similar weight loss for all three harvests. While the respiration rate of these cultivars was not measured, it has been shown previously that strawberries grown at higher temperatures had an increased respiration rate (Barrios et al., 2014). Also, discussed earlier in Chapter 3, Adhikary et al. (2020) showed that in pears, a higher respiration rate correlates with an increase in polyphenol oxidase (PPO) activity (Adhikary et al., 2021).

Individual Polyphenols

Strawberry cultivars Brilliance and Pearl vary drastically from each other not only in terms of visual appearance but also in terms of individual polyphenol profiles. Looking at the chromatograms shown in Figures 6 to 9 as examples, it is evident that the polyphenol profiles of these two strawberry cultivars are significantly different. However, there were some differences in the polyphenol profiles of Brilliance and Pearl, depending on the harvest time (Tables 6-8). Several studies have previously shown significant differences in individual polyphenol profiles between cultivars and that preharvest conditions may also contribute to the variability (Kim et al., 2012; Kim et al., 2015; Nour et al., 2017). Therefore, in the first harvest, Brilliance had significantly higher levels of most polyphenols than Pearl except for kaempferol 3-glucoside, which was considerably higher in Pearl than in Brilliance (Table 6). Also, there was no significant difference in the concentrations of kaempferol, myricetin, gallic and chlorogenic acids between Brilliance and Pearl, whereas no p-coumaric acid was detected in Pearl. The differences were more subtle in the second harvest except for pelargonidin 3-glucoside, which was significantly higher in Brilliance than in Pearl (Table 7). These results were expected since pelargonidin-3-glucoside is the primary determinant of strawberry red color (Nunes, 2015). Also higher in Brilliance than Pearl were the polyphenols kaempferol,

epicatechin, and ferulic acid. Pearl had significantly higher kaempferol 3-glucoside and gallic, chlorogenic, and ellagic acids (Table 7). There was no significant difference in the concentrations of quercetin 3-glucoside, quercetin, myricetin, and p-coumaric acid between Brilliance and Pearl. In the third harvest, results were similar to those from the second harvest (Table 8). There was no significant difference in the levels of anthocyanidins and flavanols between Brilliance and Pearl. Also, there was no significant difference in the levels of kaempferol, quercetin 3-glucoside, myricetin, and chlorogenic acid between cultivars. The concentrations of quercetin and p-coumaric, ferulic, and ellagic acid acids were higher in Brilliance than Pearl (Table 8). Overall, the levels of the anthocyanins pelargonidin 3- glucoside and cyanidin 3-glucoside were consistently higher in Brilliance than in Pearl, which agrees with data obtained for total anthocyanin content (Fig. 30). However, Pear had higher kaempferol 3-glucoside, gallic, chlorogenic, and ellagic acid levels than Brilliance, depending on the harvest. Similarly, Simirgiotis et al. (2009) reported that the ellagic acid content was about 11 times higher in the white than in the red strawberry cultivar ‘Chandler’.

After 9 days of cold storage, the levels of some polyphenol compounds decreased while others tended to increase. For Brilliance, in the first harvest, the decrease in the polyphenols pelargonidin, pelargonidin-3glucoside, quercetin 3-glucoside, ferulic and gallic acids acid was highly significant. On the other hand, cyanidin 3-glucoside, epicatechin significantly increased in Brilliance after 9 days of cold storage (Table 6). In the second harvest, the trend was somehow different. The highest decrease was observed for pelargonidin 3-glucoside, kaempferol, kaempferol 3-glucoside, myricetin, gallic and ellagic acids. The most significant increase was noted for pelargonidin, quercetin, p-coumaric, and ferulic acids (Table 7). In the third harvest, differences between days 0 and 9 were more subtle. A significant decrease was observed after 9 days of cold storage for pelargonidin, kaempferol, quercetin 3-glucoside, catechin, epicatechin, and gallic acid, while ferulic acid significantly increased. For Pearl harvested in January, there was a significant decrease in most of the polyphenols identified except for cyanidin, pelargonidin, quercetin, epicatechin, and chlorogenic acid, whose levels did not change or increased very slightly (Table 6). In the second and third harvests, all polyphenols showed a significant decrease except for cyanidin 3-glucoside and quercetin, which significantly increased after cold storage (Tables 7 and 8).

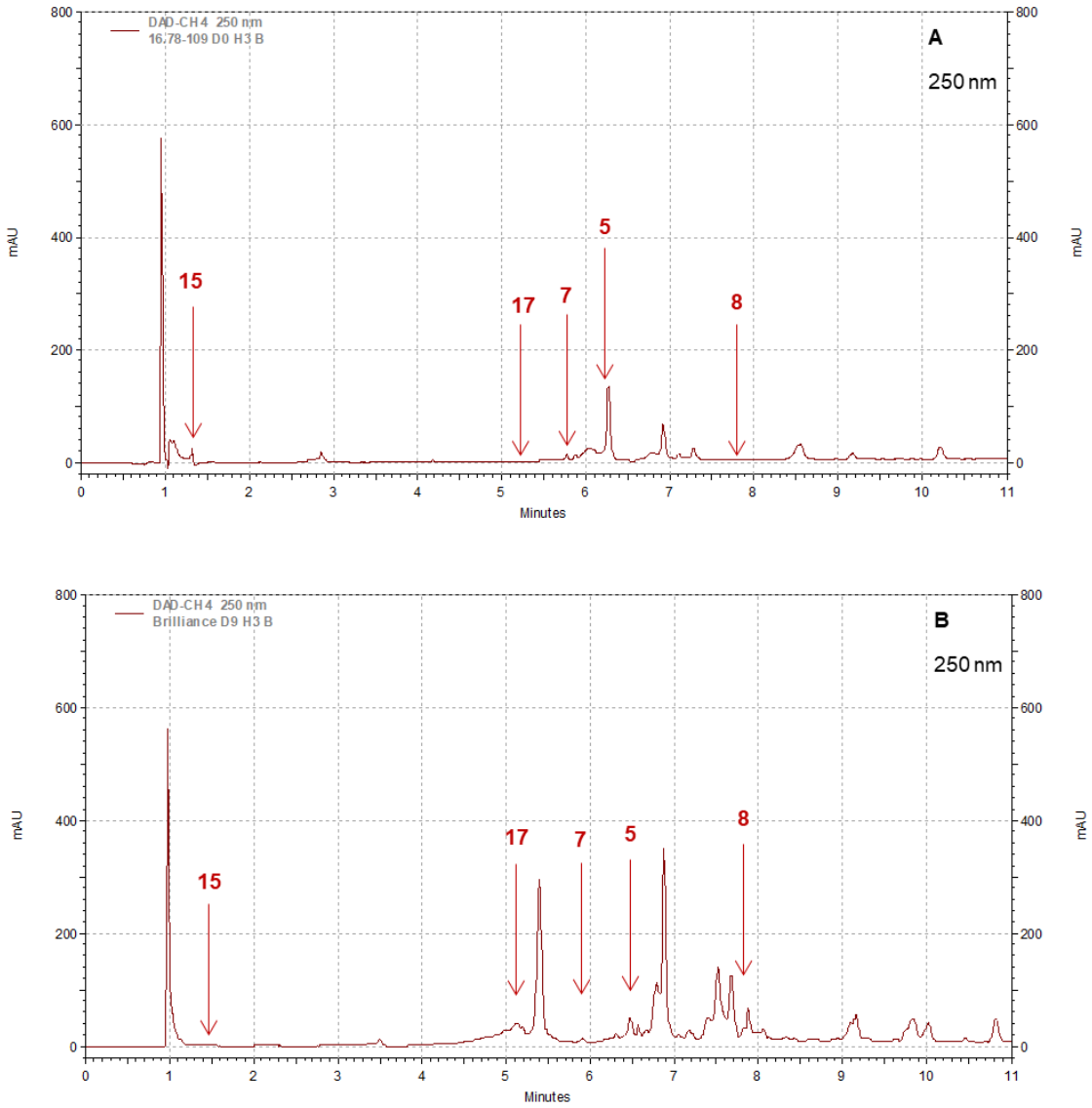

Figure 31. HPLC Chromatograms at 250nm for Brilliance and Pearl. Example of HPLC chromatograms for individual polyphenols for Pearl (**A**) and Brilliance (**B**) strawberries harvested in March and measured after 9 days at 1 °C. Wavelength 250 nm. Peak numbers: 5) kaempferol 3-glucoside, 7) quercetin 3-glucoside, 8) quercetin, 15) gallic acid, and 17) ellagic acid.

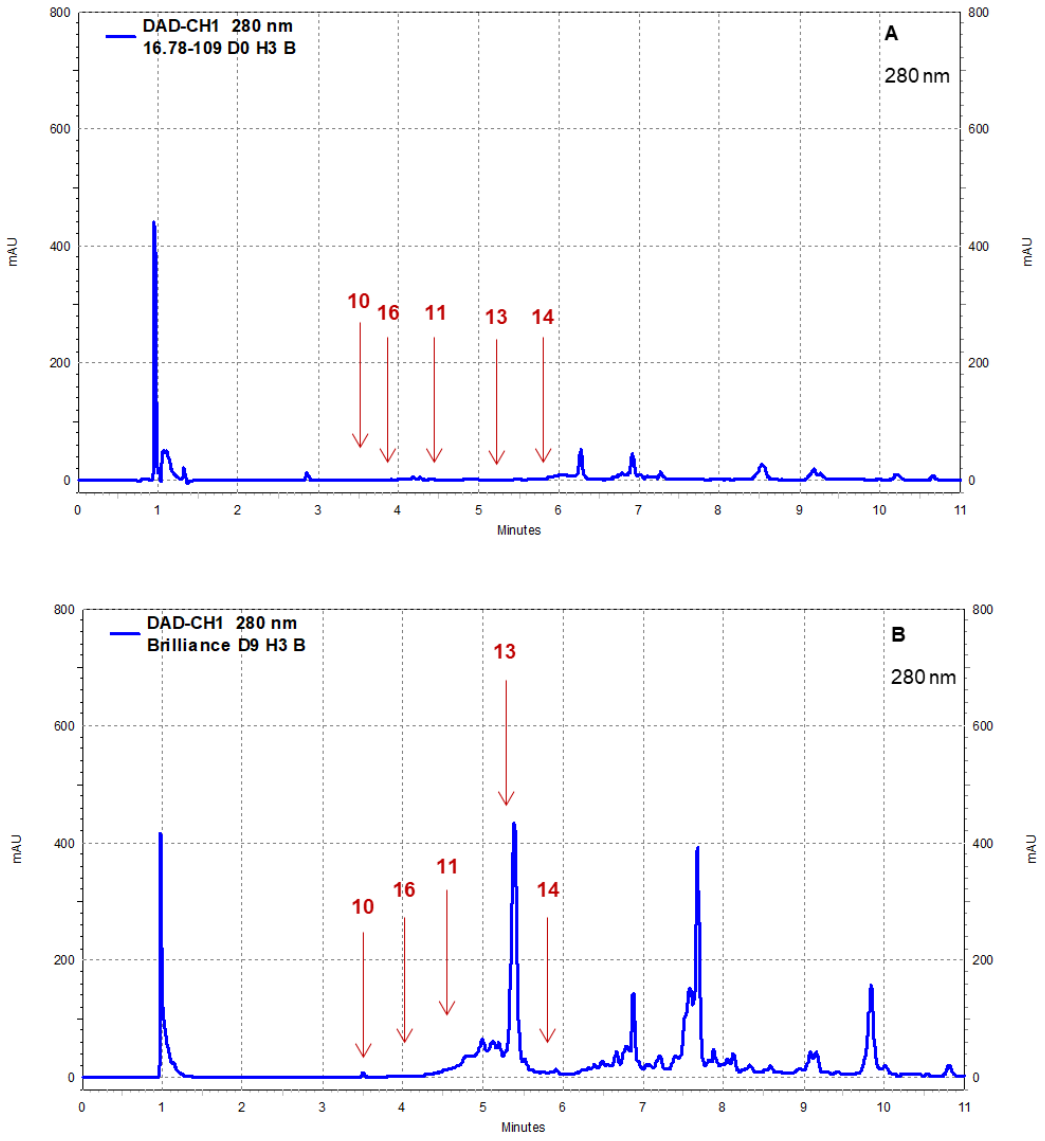

Figure 32. HPLC Chromatograms at 280nm for Brilliance and Pearl. Example of HPLC chromatograms for individual polyphenols for Pearl (**A**) and Brilliance (**B**) strawberries harvested in March and measured after 9 days at 1 °C. Wavelength 280 nm (**A**) and 520 nm (**B**). Peak numbers: 10) catechin, 11) epicatechin, 13) *p*-coumaric acid, 14) ferulic acid, and 16) chlorogenic acid.

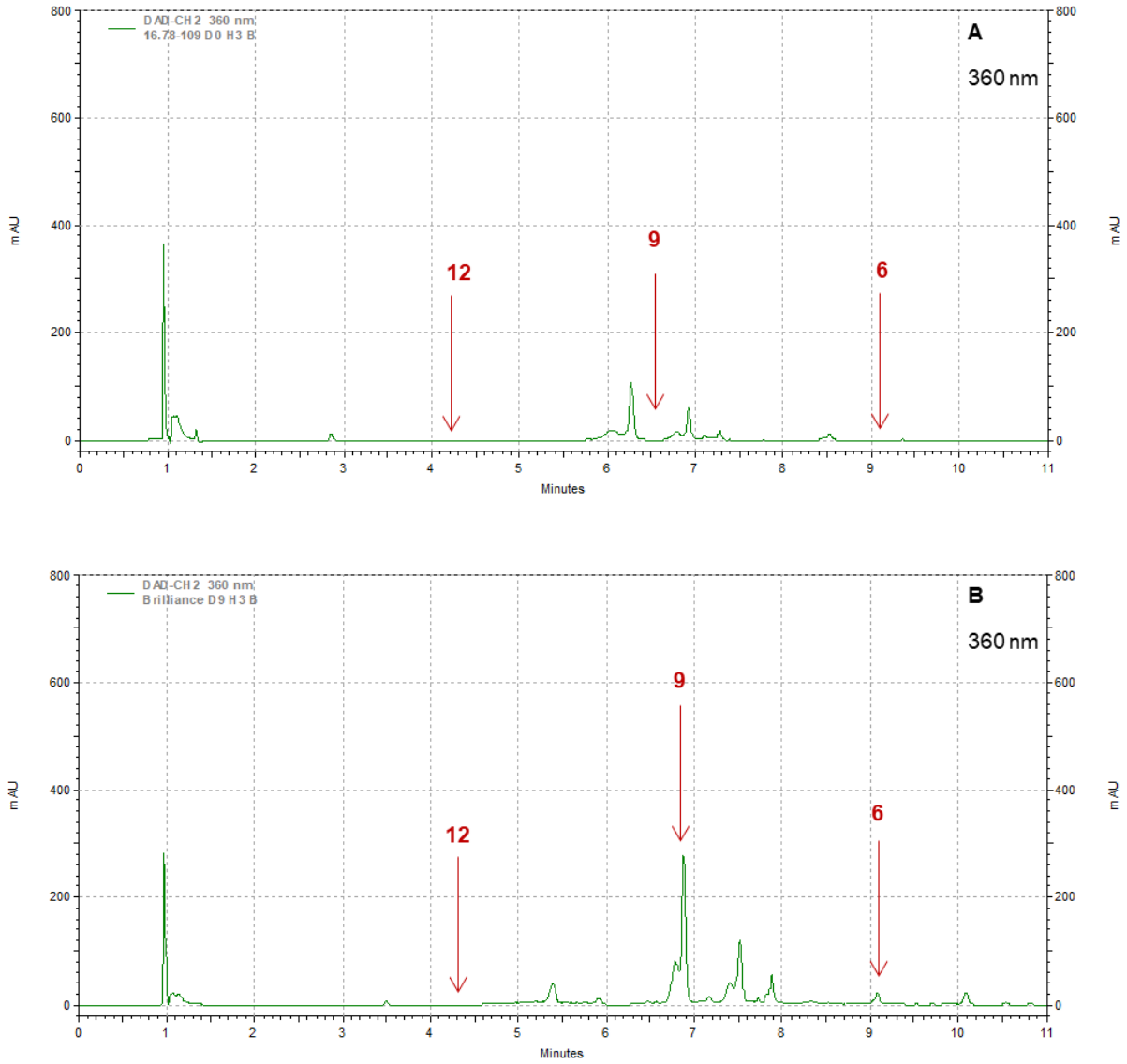

Figure 33. HPLC Chromatograms at 360nm for Brilliance and Pearl. Example of HPLC chromatograms for individual polyphenols for Pearl (**A**) and Brilliance (**B**) strawberries harvested in March and measured after 9 days at 1 °C. Wavelength 360 nm. Peak numbers: 6) kaempferol 9) myricetin, and 12) caffeic acid.

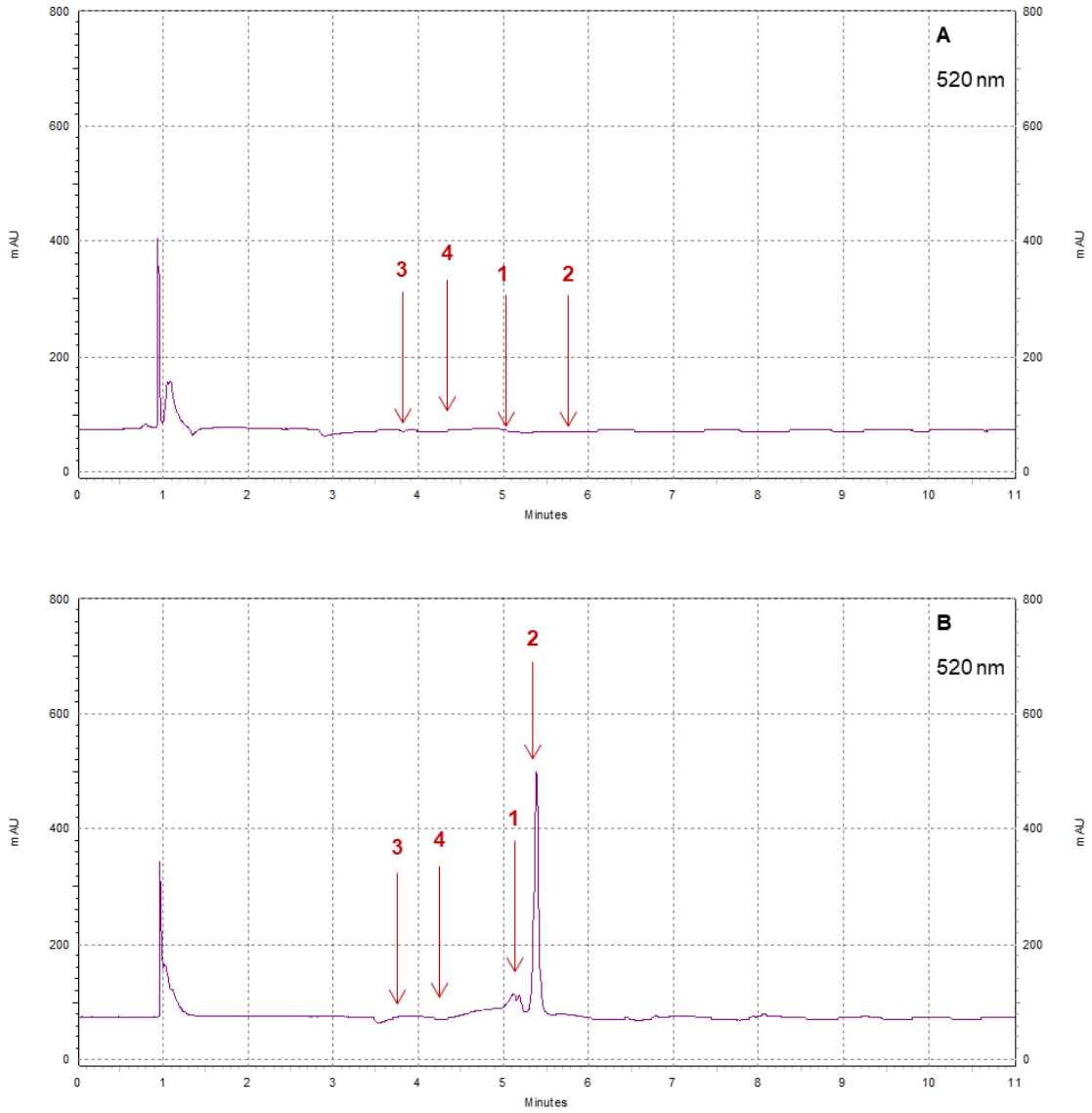

Figure 34. HPLC Chromatograms at 520nm for Brilliance and Pearl. Example of HPLC chromatograms for individual polyphenols for Pearl (**A**) and Brilliance (**B**) strawberries harvested in March and measured after 9 days at 1 °C. Wavelength 520 nm. Peak numbers: 1) cyanidin, 2) pelargonidin, 3) cyanidin-3-glucoside, and 4) pelargonidin 3-glucoside.

Table 6. The concentration (mg $100g^{-1}$ of fruit dry weight) of the major individual polyphenols in Brilliance and Pearl strawberry cultivars at harvest (day 0) and after 9 days at 1°C (January harvest).

Polyphenols	Brilliance		Pearl		
	Day 0	Day 9	Day 0	Day 9	*p* value
Anthocyanidins					
Cyanidin	15.77 ± 2.49 b[a]	12.16 ± 1.66 ab	8.77 ± 0.10 ab	4.85 ± 0.13 a	p = 0.0823
Pelargonidin	88.11 ± 4.94 a	24.68 ± 2.99 b	37.09 ± 3.50 b	28.07 ± 1.18 b	p = 0.0004
Anthocyanins					
Cyanidin 3-glucoside	3.81 ± 2.13 ac	7.58 ± 0.00 c	0.00 ± 0.00 a	12.68 ± 0.00 b	p = 0.0091
Pelargonidin 3-glucoside	135.47 ± 0.00 a	10.60 ± 0.00 d	32.42 ± 8.77 c	43.28 ± 0.00 b	p < 0.0001
Flavonols					
Kaempferol 3-glucoside	16.01 ± 2.63 b	12.73 ± 1.59 b	79.11 ± 9.78 a	24.75 ± 1.63 b	p = 0.0007
Kaempferol	12.31 ± 0.53 a	11.59 ± 0.16 a	10.81 ± 1.34 a	5.18 ± 0.22 b	p = 0.0018
Quercetin 3-glucoside	41.01 ± 4.08 a	9.16 ± 0.11 b	16.78 ± 5.84 b	5.01 ± 1.06 b	p = 0.0053
Quercetin	5.19 ± 1.02 a	4.37 ± 0.31 a	0.82 ± 0.81 b	0.23 ± 0.12 b	p = 0.0129
Myricetin	24.35 ± 4.31 a	21.35 ± 7.22 a	22.76 ± 2.09 a	5.86 ± 0.06 b	p = 0.068
Flavanols					
Catechin	13.29 ± 0.00 a	6.51 ± 0.00 c	10.46 ± 0.00 b	8.18 ± 0.59 c	p = 0.0003
Epicatechin	22.23 ± 0.11 b	45.79 ± 2.10 a	10.58 ± 0.16 c	10.73 ± 1.47 c	p = 0.0001
Phenolic acids					
Caffeic Acid	ND[b]	ND	ND	ND	N/A
p-Coumaric Acid	22.17 ± 1.31 a	26.39 ± 4.60 a	ND	ND	p = 0.0037
Ferulic Acid	73.11 ± 5.99 a	7.85 ± 0.07 b	13.96 ± 1.94 b	3.85 ± 0.54 b	p = 0.0001
Gallic Acid	14.10 ± 1.59 a	6.42 ± 0.22 b	16.50 ± 0.00 a	5.50 ± 0.07 b	p = 0.0017
Chlorogenic Acid	2.55 ± 0.00 a	1.25 ± 0.00 a	2.01 ± 0.00 a	3.38 ± 1.39 a	p = 0.3677
Hydrolyzable tannins					
Ellagic Acid	18.46 ± 3.30 b	19.30 ± 3.67 b	10.42 ± 0.74 ab	4.65 ± 0.05 a	p = 0.0474

[a] Letters after averages denote significant differences ($p < 0.05$) between treatments based on Tukey's HSD test; averages followed by the same letter are not significantly different.
[b] ND = non-detected.

Table 7. The concentration (mg $100g^{-1}$ of fruit dry weight) of the major individual polyphenols in Brilliance and Pearl strawberry cultivars at harvest (day 0) and after 9 days at 1°C (February harvest).

Polyphenols	Brilliance		Pearl		
	Day 0	Day 9	Day 0	Day 9	*p* value
Anthocyanidins					
Cyanidin	11.83 ± 0.10 a[a]	8.15 ± 1.25 b	11.44 ± 0.34 a	6.76 ± 0.57 b	p = 0.0045
Pelargonidin	54.55 ± 3.56 b	152.30 ± 18.48 a	45.54 ± 4.23 b	27.89 ± 4.31 b	p = 0.0054
Anthocyanins					
Cyanidin 3-glucoside	ND	ND	ND	10.48 ± 0.00 a	p < 0.0001
Pelargonidin 3-glucoside	215.63 ± 11.05 a	1.87 ± 0.00 b	17.64 ± 0.16 b	2.45 ± 0.00 b	p < 0.0001
Flavonols					
Kaempferol 3-glucoside	23.05 ± 6.04 b	6.48 ± 0.65 b	100.15 ± 5.96 a	5.00 ± 0.86 b	p = 0.0005
Kaempferol	20.44 ± 0.88 a	7.92 ± 0.94 c	13.48 ± 0.79 b	6.32 ± 0.45 c	p = 0.0016
Quercetin 3-glucoside	62.03 ± 0.00 b	81.98 ± 9.62 a	53.70 ± 0.00 b	4.42 ± 0.84 c	p = 0.0004
Quercetin	0.36 ± 0.19 c	9.63 ± 0.00 a	0.54 ± 0.49 c	4.18 ± 1.01 b	p = 0.0002
Myricetin	19.75 ± 1.58 a	7.94 ± 0.75 b	21.90 ± 2.94 a	6.59 ± 0.32 b	p = 0.0039
Flavanols					
Catechin	60.90 ± 11.51 a	6.08 ± 0.23 b	59.03 ± 13.74 a	8.61 ± 1.88 b	p = 0.006
Epicatechin	317.50 ± 31.35 a	5.69 ± 1.52 b	17.03 ± 4.26 b	4.22 ± 2.33 b	p < 0.0001
Phenolic acids					
Caffeic Acid	ND[b]	ND	ND	ND	N/A
p-Coumaric Acid	13.30 ± 0.03 ab	23.54 ± 3.56 b	13.06 ± 6.03 ab	2.47 ± 0.40 a	p = 0.084
Ferulic Acid	37.58 ± 4.25 a	50.65 ± 5.46 a	14.50 ± 0.00 b	4.85 ± 0.51 b	p = 0.0018
Gallic Acid	74.07 ± 2.78 b	6.68 ± 0.71 c	92.60 ± 3.33 a	7.42 ± 1.10 c	p < 0.0001
Chlorogenic Acid	2.74 ± 0.00 a	1.17 ± 0.04 a	5.01 ± 1.38 a	4.85 ± 2.84 a	p = 0.4327
Hydrolyzable tannins					
Ellagic Acid	13.64 ± 0.19 ab	7.47 ± 0.86 ab	18.57 ± 4.51 b	5.44 ± 0.06 a	p = 0.0678

[a] Letters after averages denote significant differences ($p < 0.05$) between treatments based on Tukey's HSD test; averages followed by the same letter are not significantly different.
[b] ND = non-detected.

Table 8. The concentration (mg $100g^{-1}$ of fruit dry weight) of the major individual polyphenols in Brilliance and Pearl strawberry cultivars at harvest (day 0) and after 9 days at 1°C (March harvest).

Polyphenols	Brilliance		Pearl		
	Day 0	Day 9	Day 0	Day 9	*p* value
Anthocyanidins					
Cyanidin	15.40 ± 2.20 a	14.28 ± 5.08 a	13.26 ± 1.06 a	6.46 ± 0.61 a	p = 0.2101
Pelargonidin	63.14 ± 0.00 a	15.22 ± 7.06 b	55.91 ± 5.86 a	24.11 ± 2.34 b	p = 0.0024
Anthocyanins					
Cyanidin 3-glucoside	4.07 ± 0.00 a	3.57 ± 0.00 a	0.01 ± 0.01 a	5.65 ± 2.28 a	p = 0.2036
Pelargonidin 3-glucoside	38.11 ± 0.00 a	32.79 ± 0.00 a	18.98 ± 3.62 b	3.42 ± 0.00 c	p = 0.0008
Flavonols					
Kaempferol 3-glucoside	14.32 ± 1.87 c	20.22 ± 3.58 c	119.69 ± 6.78 a	54.29 ± 1.54 b	p < 0.0001
Kaempferol	14.08 ± 1.29 b	7.40 ± 1.68 ab	13.08 ± 0.23 b	5.65 ± 0.42 a	p = 0.0491
Quercetin 3-glucoside	32.54 ± 8.20 a	6.02 ± 1.16 a	24.37 ± 6.13 a	9.85 ± 1.92 a	p = 0.113
Quercetin	17.44 ± 4.48 b	14.05 ± 3.61 ab	0.93 ± 0.55 c	2.82 ± 2.00 ac	p = 0.0863
Myricetin	16.20 ± 1.10 a	17.50 ± 11.40 a	23.14 ± 4.65 a	6.10 ± 0.00 a	p = 0.5983
Flavanols					
Catechin	14.71 ± 0.00 b	6.28 ± 0.93 a	15.14 ± 0.00 b	8.59 ± 2.52 ab	p = 0.0221
Epicatechin	28.78 ± 6.34 a	3.96 ± 0.01 a	22.14 ± 5.68 a	31.22 ± 9.49 a	p = 0.2178
Phenolic acids					
Caffeic Acid	ND	ND	ND	ND	N/A
p-Coumaric Acid	14.13 ± 1.27 a	15.24 ± 1.36 a	0.15 ± 0.15 b	1.16 ± 0.61 b	p = 0.0002
Ferulic Acid	37.58 ± 13.38 b	72.40 ± 11.20 ab	6.22 ± 0.68 a	5.96 ± 1.06 a	p = 0.0184
Gallic Acid	14.11 ± 0.45 b	5.05 ± 0.15 c	59.87 ± 3.86 a	5.53 ± 0.13 c	p < 0.0001
Chlorogenic Acid	2.83 ± 0.00 a	2.80 ± 1.14 a	2.91 ± 0.00 a	1.14 ± 0.03 a	p = 0.2629
Hydrolyzable tannins					
Ellagic Acid	18.04 ± 3.18 b	17.44 ± 1.80 b	12.73 ± 0.18 ab	6.20 ± 0.17 a	p = 0.0521

[a] Letters after averages denote significant differences ($p < 0.05$) between treatments based on Tukey's HSD test; averages followed by the same letter are not significantly different.
[b] ND = non-detected.

After 9 days of cold storage and compared to Brilliance harvested in January, Pearl had similar levels of pelargonidin, catechin, and chlorogenic acid but significantly higher levels of pelargonidin 3-glucoside, kaempferol 3-glucoside than Brilliance. The remaining polyphenols were considerably higher in Brilliance than Pearl after cold storage (Table 6). In the second harvest, levels of quercetin 3-glucoside, quercetin, p-coumaric acid, and ferulic acid were significantly higher in Brilliance than in Pearl after cold storage (Table 7). Finally, in the third harvest, concentrations of cyanidin, pelargonidin 3-glucoside, quercetin, myricetin, caffeic, coumaric, and ellagic acids were significantly lower in Pearl than in Brilliance. In contrast, kaempferol 3-glucoside and epicatechin were significantly higher in Pearl than in Brilliance after 9 days of cold storage (Table 8). Like the results obtained for Radiance and Sensation, when strawberries have greater epicatechin concentrations, there was either very little or a non-detectible amount of cyanidin-3-glucoside (see Chapter 4). These results agree with those previously reported in the literature. That is since epicatechin and cyanidin-3-glucoside are products of cyanidin, one of the compounds will likely be present in higher amounts than the other (Fischer et al., 2014). Besides, pelargonidin and kaempferol are both derived from dihydrokaempferol. Therefore, the close to opposite concentrations of pelargonidin-3-glucoside and kaempferol-3-glucoside within a strawberry would be expected. Pearl has a mutation that knocks down the synthesis of anthocyanins and specifically pelargonidin-3-glucoside. It would explain that almost all the dihydrokaempferol within the fruit would be converted into kaempferol and then kaempferol-3-glucoside.

Polyphenol Classes

Polyphenols identified in strawberries were grouped into major classes: anthocyanidins (cyanidin plus pelargonidin), anthocyanins (cyanidin 3-glucoside plus pelargonidin 3-glucoside), flavonols (quercetin, kaempferol, quercetin 3-glucoside, kaempferol 3-glucoside, plus myricetin), flavanols (catechin plus epicatechin), phenolic acids (p-coumaric acid, ferulic acid, caffeic acid, gallic acid, plus chlorogenic acid) and hydrolyzable tannins (ellagic acid).

Although there was some variability in the levels of each polyphenol group depending on the date of harvest, on average at harvest (day 0), Brilliance had higher (first harvest) or similar (second and third harvests) levels of anthocyanidins than Pearl (Fig. 35). After storage, the levels of anthocyanidins significantly decreased in Brilliance, except for the second harvest, where there was an unexpected

increase, most likely due to an error in sample processing (Fig 35). However, there was not a significant decrease in the anthocyanidin content of Pearl after 9 days of storage.

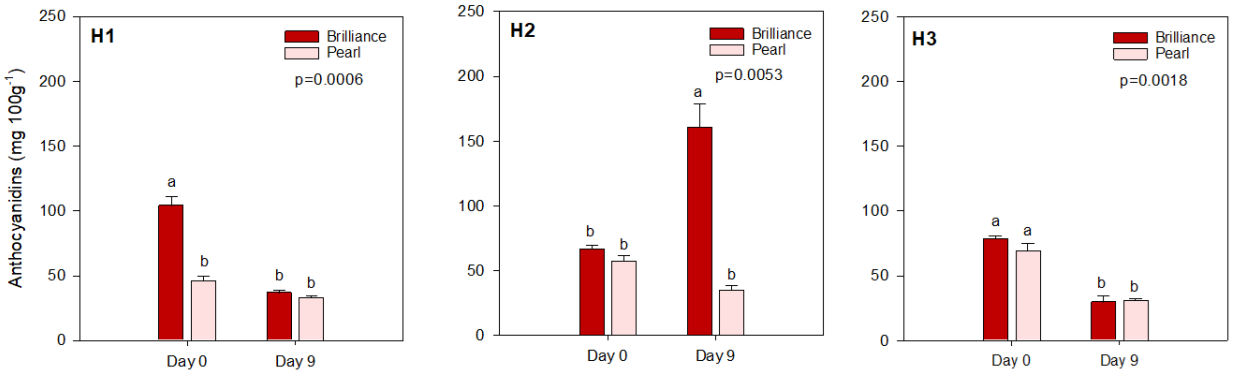

Figure 35. Anthocyanidin Content from HPLC Analysis for Brilliance and Pearl. Anthocyanidin content of strawberries Brilliance and Pearl at the day of harvest (Day 0) and after 9 days of storage at 1°C. H1) First harvest in January; H2) Second harvest in February; H3) Third harvest in March. Letters above each bar denote significant differences ($p < 0.05$) between treatments based on Tukey's HSD test; bars with the same letter are not significantly different.

Anthocyanins were significantly higher in Brilliance than in Pearl, particularly in the two first harvests (Fig. 36). The levels were very high at harvest in Brilliance and declined dramatically after cold storage. After cold storage, the decrease was significantly higher in Brilliance than in Pearl, particularly in the second harvest.

For the flavonols, Pearl had, for all three harvests, a significantly greater concentration at harvest (Fig. 37). These results were most likely due to Pearl having a significantly greater concentration of kaempferol-3-glucoside than Brilliance (Tables 6-8). However, there was a decrease in flavonol content for both cultivars after 9 days of storage, but Pearl had a more significant reduction than Brilliance.

At harvest, there was more significant variability in the levels of flavanols for Brilliance than for Pearl (Fig. 38). Brilliance had slightly higher (first harvest), significantly higher (second harvest), or similar (third harvest) concentrations of flavanols than Pearl. Particularly in the second harvest, the levels of flavonols in Brilliance were more than eight times higher than for the first and third harvests and significantly higher than in Pearl. After 9 days of cold storage, flavanol concentration decreased in Brilliance from the second and third harvests. However, there was a slight increase in flavanol concentration after storage in the first

harvest. Results obtained for Pearl were more consistent across harvests, but there was not a significant decrease in the flavanol content after cold storage (Fig. 38).

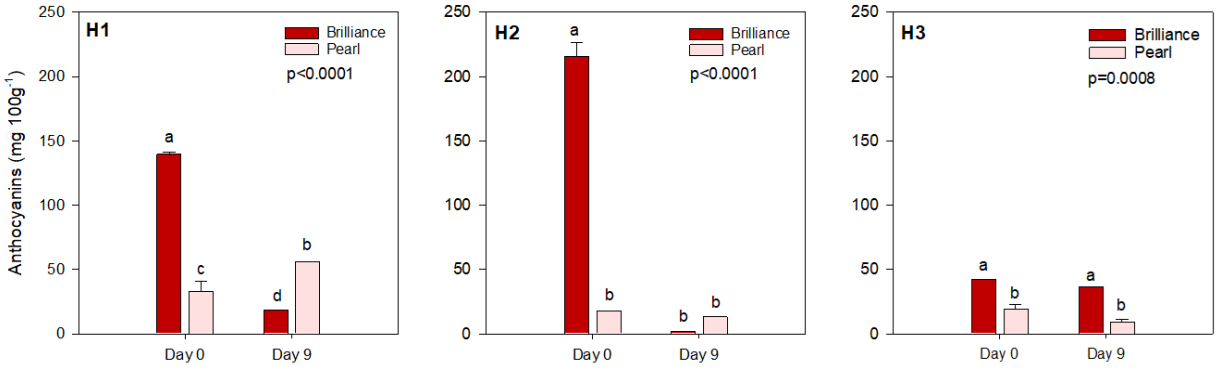

Figure 36. Anthocyanin Content from HPLC Analysis for Brilliance and Pearl. Anthocyanin content of strawberries Brilliance and Pearl at the day of harvest (Day 0) and after 9 days of storage at 1°C. H1) First harvest in January; H2) second harvest in February; H3) Third harvest in March. Letters above each bar denote significant differences ($p < 0.05$) between treatments based on Tukey's HSD test; bars with the same letter are not significantly different.

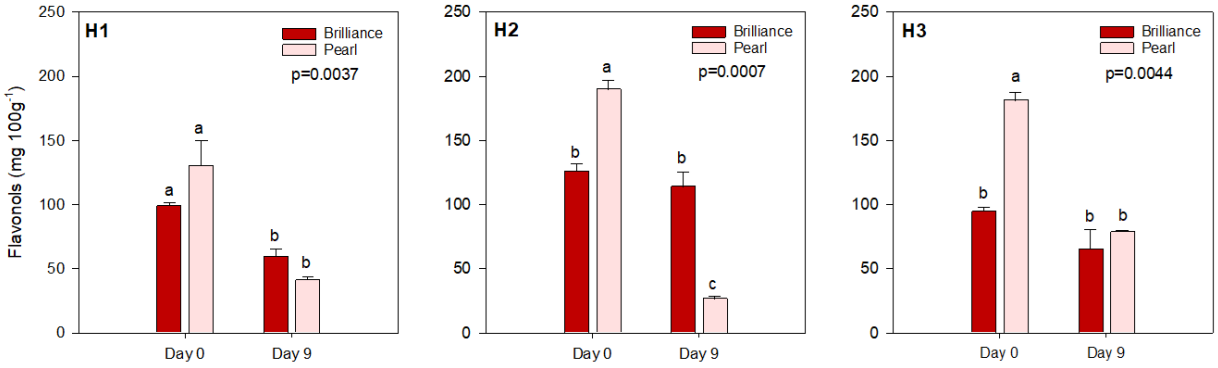

Figure 37. Flavonol Content from HPLC Analysis for Brilliance and Pearl. Flavonol content of strawberries Brilliance and Pearl at the day of harvest (Day 0) and after 9 days of storage at 1°C. H1) First harvest in January; H2) Second harvest in February; H3) Third harvest in March. Letters above each bar denote significant differences ($p < 0.05$) between treatments based on Tukey's HSD test; bars with the same letter are not significantly different.

For the phenolic acids, there was a significant difference between Brilliance and Pearl on the first harvest, where Brilliance had higher levels of phenolic acids than Pearl (Fig. 39). However, phenolic acid levels at harvest were similar for both cultivars on the second and third harvests. After cold storage, the

levels of phenolic acids significantly decreased for both cultivars, regardless of the harvest time. However, the decrease in phenolic acid was more dramatic in Pearl than Brilliance.

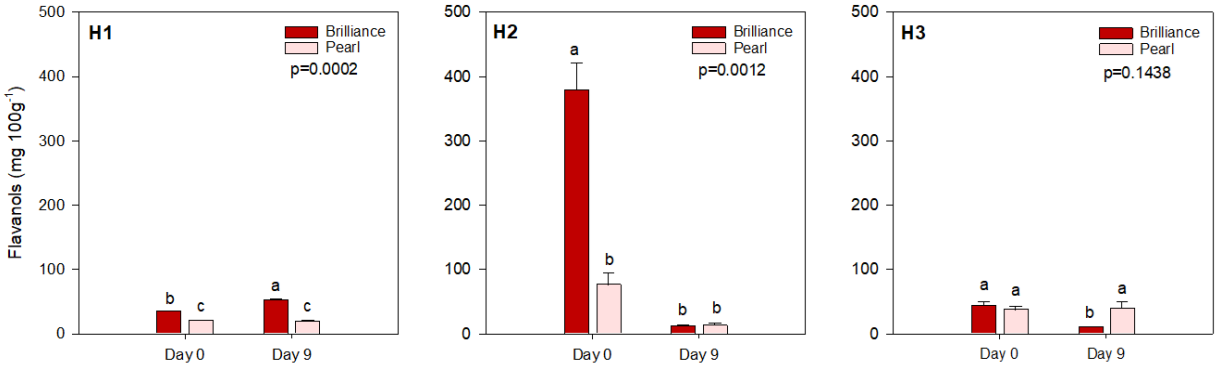

Figure 38. Flavanol Content from HPLC Analysis for Brilliance and Pearl. Flavanol content of strawberries Brilliance and Pearl at the day of harvest (Day 0) and after 9 days of storage at 1°C. H1) First harvest in January; H2) second harvest in February; H3) Third harvest in March. Letters above each bar denote significant differences ($p < 0.05$) between treatments based on Tukey's HSD test; bars with the same letter are not significantly different.

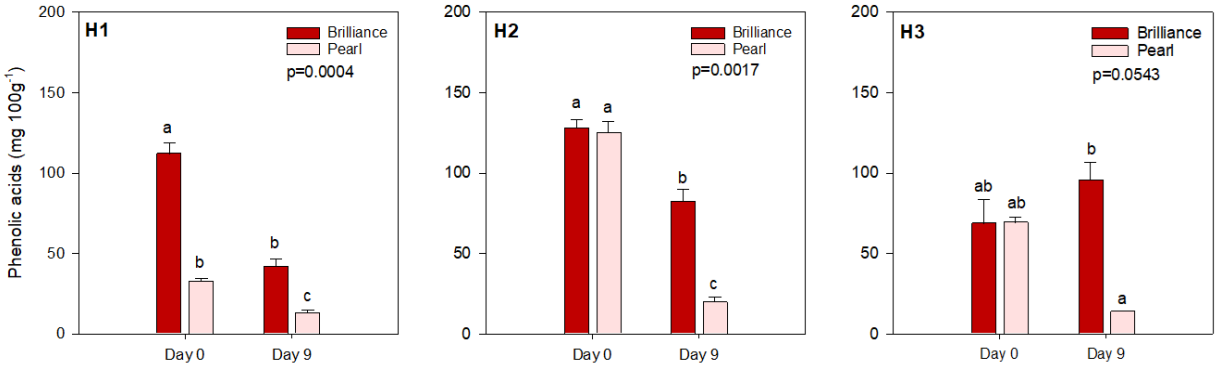

Figure 39. Phenolic Acid Content from HPLC Analysis for Brilliance and Pearl. The phenolic acid content of strawberries Brilliance and Pearl at the day of harvest (Day 0) and after 9 days of storage at 1°C. H1) First harvest in January; H2) Second harvest in February; H3) Third harvest in March. Letters above each bar denote significant differences ($p < 0.05$) between treatments based on Tukey's HSD test; bars with the same letter are not significantly different.

Finally, the hydrolyzable tannins (comprised of only ellagic acid) at harvest were higher in Brilliance than in Pearl in the first and third harvests. Still, the opposite was observed in the second harvest (Fig. 40). After cold storage, there was a decrease in the levels of ellagic acid in Pearl, while in Brilliance, they were similar after storage.

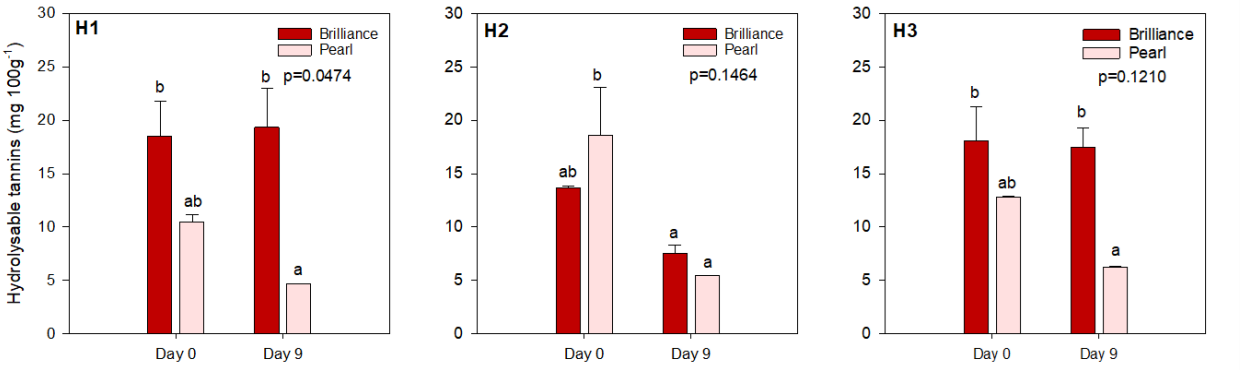

Figure 40. Hydrolyzable Tannin Content from HPLC Analysis for Brilliance and Pearl. Hydrolyzable tannins content of strawberries Brilliance and Pearl at the day of harvest (Day 0) and after 9 days of storage at 1°C. H1) First harvest in January; H2) Second harvest in February; H3) Third harvest in March. Letters above each bar denote significant differences ($p < 0.05$) between treatments based on Tukey's HSD test; bars with the same letter are not significantly different.

Overall, there was a significant difference between Brilliance and Pearl regarding polyphenol classes and their concentrations. There was also a considerable difference between harvests due to the difference in weather conditions during growing. Pearl seems to be more susceptible to sun exposure and higher field temperatures that accompany sun exposure. These could be significant factors in the observed fluctuation in polyphenols between harvests. It has been shown before that there is a considerable difference in anthocyanin and total phenolic contents between different strawberry cultivars and variability in response to UV light exposure in terms of polyphenol accumulation (Cervantes et al., 2019; Palmieri et al., 2017).

Conclusions

This study showed that the white-fruited strawberry Pearl has a significantly different polyphenolic profile than the commercial standard red-fruited strawberry cultivar Brilliance. At harvest, Pearl has lower pelargonidin 3-glucoside but higher kaempferol 3-glucoside, gallic, chlorogenic, and ellagic acids than Brilliance. After postharvest storage, Pearl had lower water loss compared to Brilliance and consequently showed a less dramatic decline in individual polyphenols. However, Pearl seems highly sensitive to increased temperatures and high UV radiation, resulting in increased anthocyanin synthesis as a protective response. The increase in total anthocyanin contents, particularly in the third harvest, where the temperatures and UV radiation were higher, resulted in fruit with a noticeable pink blush and not creamy

white. Thus, if commercialized as a white or pearl strawberry, the plant and fruit would benefit from UV protection during growth.

CHAPTER 6: GENERAL CONCLUSIONS

Pre- and post-harvest conditions significantly affect the overall quality of strawberry fruit and impact their polyphenol profiles specifically. Overall, results presented in this thesis showed the significant impact of pre-harvest weather conditions on the biochemical attributes of strawberries, with rainfall affecting the metabolism of the fruit and accelerating biochemical breakdown. Results also showed that exposure of strawberries to 20°C creates stress on the fruit, demonstrated by a highly significant decrease in all biochemical markers of quality measured, comparatively to storage at 1°C or 10°C (Chapter 3). Temperature stress also significantly impacts the individual polyphenol profiles of 'Florida Radiance' and Sweet Sensation ® 'FL127' strawberry cultivars, particularly the concentrations of pelargonidin-3-glucoside and epicatechin (Chapter 4). Furthermore, results show the significant differences in polyphenol profiles between cultivars, especially between red and white-fruited strawberries. The white-fruited cultivar 'Florida Pearl' has less pelargonidin-3 glucoside (red pigment) but a significantly higher amount of kaempferol-3-glucoside.

Future studies regarding cultivar differences and their response to pre and postharvest stress should focus on the molecular mechanisms behind stress response. For example, studying the enzymatic activity of major enzymes on the phenylpropanoid pathway such ad phenylalanine-ammonia lyase (PAL), chalcone synthase (CHS), flavonol synthase (FLS), and polyphenol oxidase (PPO). With the significant difference in concentrations of kaempferol 3-glucoside content between red and white-fruited strawberries, investigating the activity of FLS could help with further understanding on how white strawberries react to stress compared to their red-fruited strawberry counterparts. Additional work should also focus on the effect of long-term freezing storage on the polyphenol content of strawberries. That is, how freezing time-temperature combinations affect the biochemical make-up of the strawberry fruit. The overall quality appearance of 'Florida Pearl' seems to depend on UV radiation when the fruit is grown in the open field. Thus, optimal growing and postharvest conditions for the white-fruited strawberry should be better studied as they would provide critical information for the future commercialization of this cultivar.

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