Composition of Commercial Strawberry Cultivars and Advanced Selections as Affected by Season, Harvest, and Postharvest Storage

Yanina Perez Cayo

University of Florida, Gulf Coast Research and Education Center, Wimauma, FL 33598

Steven Sargent

Horticultural Sciences Department, University of Florida, P.O. Box 110690, IFAS, Gainesville, FL 32611-0690

Cecilia do Nascimento Nunes¹

University of South Florida, Department of Cell Biology, Microbiology and Molecular Biology, 4202 E. Fowler Ave. ISA 2015, Tampa, FL 33620

Vance Whitaker

University of Florida, Gulf Coast Research and Education Center, Wimauma, FL 33598

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Abstract. Strawberries are one of the most important food crops grown in Florida, with a harvested area of ≈10,000 acres. The University of Florida strawberry breeding program develops cultivars adapted to this region and its particular weather conditions, with a major aim of increasing overall quality. The objective of this study was to compare the fruit of advanced breeding selections to those of commercial cultivars, for compositional attributes. Seven different strawberry genotypes were compared at harvest and after 7 days at 4 °C across multiple harvest dates during two consecutive years. Compositional attributes were highly influenced by year, harvest date within a year, genotype, and storage. Overall, compared with other genotypes, selection FL 09-127 exhibited consistently higher soluble solids and total sugar (TS) contents at harvest and after cold storage. Higher ascorbic acid (AA) and phenolic contents at harvest were observed in selection FL 07-193. However, its anthocyanin content was among the lowest. In contrast, FL 10-47 exhibited relatively low AA content at harvest but consistently high total anthocyanins (TACs) and total phenolic (TP) contents after storage. Overall, results from this study provide valuable information to the breeding process by identifying new genotypes with improved compositional attributes combined with suitable quality characteristics after cold storage.

Strawberry (*Fragaria × ananassa*) fruit is one of the most important commodities produced in Florida. According to the USDA-ARS database, Florida, produced around 207 million pounds of strawberries in 2014, with an estimated return to the grower of ≈\$306 million (USDA, 2015). University of Florida strawberry cultivars are developed for adaptation to a mild winter and early spring (December to March) climate. High and consistent fruit quality across years and harvests within years is an important goal of the breeding program. Toward this goal, the breeding program seeks to quantify and

enhance important chemical components in strawberry fruit that may impact appearance, flavor, and nutritional value.

Sugars, acids, and polyphenols contribute significantly to flavor quality, whereas anthocyanins contribute to the color of the fruit. Vitamin C is an important contributor to the nutritional value of strawberry as it is available in significant amounts in the fruit. In Florida, large variations in the sensory characteristics of several commercial genotypes were reported over a 2-year period and were related to variations in the soluble solids content (SSC)/acidity ratios between strawberry cultivars (Jouquand et al., 2008). In addition, preharvest factors (e.g., weather, disease control treatments), year of harvest, harvest time, maturity of the fruit, and postharvest factors such as temperature and humidity have also had a significant impact on the overall

quality of strawberry fruit (Jin et al., 2011; Kafkas et al., 2007; Nunes, 2008; Pozo-Insfran et al., 2006; Schwieterman et al., 2014; Tulipani et al., 2011).

Both environmental and cultivar-specific characteristics are important for the accumulation of sugars and organic acids (Kafkas et al., 2007; Strum et al., 2003), with strong fluctuations observed from harvest-to-harvest within years (Pozo-Insfran et al., 2006). Thus, the levels of sugars and organic acids differ considerably across strawberry genotypes and have an important impact on the sensory attributes of the fruit (Jouquand et al., 2008). Sugars contribute significantly to the flavor characteristics of strawberry, and the amount present will impact the likeability of the fruit (Maas et al., 1996; Schwieterman et al., 2014). SSC is a useful measure to estimate TS content (Perkins-Veazie, 1995), and, because it can be easily and cheaply performed, is often used as a breeding screen on a large scale. Sucrose, glucose, and fructose account for more than 99% of the TSs in ripe strawberry fruit. As ripening progress, sucrose content decreases significantly, whereas glucose and fructose increase (Ferreira et al., 2007; Montero et al., 1996; Strum et al., 2003). Depending on the cultivar, fruit maturity, and environmental factors, acidity of strawberry may range from 0.45% to 1.81% (Kays, 1997; Perkins-Veazie, 1995; Sadler and Murphy, 1998). In ripe strawberries, citric acid accounts for 88% of the total organic acids, whereas malic acid is the second most abundant organic acid in the fruit (Green, 1971; Kim and Moon, 1993).

Bioactive compounds such as AA (Vitamin C) and polyphenols are also present in significant amounts in strawberry fruit. AA is considered a vital vitamin for human health and strawberries are noted for their particularly high levels (Naidu, 2003; Prottogente et al., 2002). However, there is also a great variability in the AA content (19.3 to 71.5 mg/100 g) between strawberry cultivars from different growing locations (Nelson et al., 1972). Polyphenols are abundant in strawberries and appear to have many functions in plants and vast biological potentialities (Häkkinen and Törrönen, 2000). Strawberries are consistently rated among the top sources for polyphenolic contents, showing a 2- to 11-fold larger antioxidant capacity when compared with other fruits (Halvorsen et al., 2002, 2006; Pellegrini et al., 2003; Prottogente et al., 2002; Scalzo et al., 2005). Flavonoids (particularly anthocyanins), along with flavonols and flavanols, are considered the major class of polyphenols in strawberries, followed by hydrolysable tannins and phenolic acids (Aaby et al., 2005; Kähkönen et al., 2001). Anthocyanins are the most abundant polyphenols in strawberries, particularly pelargonidin-3-glucoside which is the major anthocyanin in strawberries, comprising 80% of the TAC pool, followed by cyanidin-3-glucoside (Bridle and García-Viguera, 1997; Clifford, 2000; Lopes-da-Silva et al., 2002). The concentration of anthocyanins in the epidermis and cortex of strawberries is primarily responsible

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¹Corresponding author. E-mail: mariacecilia@usf.edu.

for the vivid red color of the fruit (Timberlake and Bridle, 1982). Anthocyanin content in strawberry fruit is highly correlated with the color of the fruit. Thus, color has been considered a good screening tool for high anthocyanin strawberries in breeding programs (Fredericks et al., 2012; Nunes, 2015), though the dark color is often undesirable in commerce due to the perception that dark fruit are overripe.

Because of the continuous interest in developing new strawberry genotypes with improved quality traits, additional data on chemical composition and postharvest behavior of new strawberry breeding selections are necessary. These data facilitate the release of new strawberry cultivars and provide valuable information that can be made available to the strawberry industry on release. The objectives of this study were to 1) compare commercial strawberry cultivars and advanced breeding selections for compositional attributes such as SSC, titratable acidity (TA), TSs, AA, phenolics, and anthocyanin contents across multiple harvest dates during two consecutive years and, 2) determine the effect of storage at 4 °C and 95% relative humidity (RH) on the compositional attributes of different strawberry genotypes from different years and harvest dates.

Material and Methods

Cultivation method, cultivar selection, harvesting, and postharvest handling. Strawberries were grown under standard commercial practices at the Gulf Coast Research and Education Center of the University of Florida in Balm, FL, during the 2011–12 and 2012–13 production years. Bare-root runner plants were planted on two-row raised beds covered with black polyethylene mulch. A mixture of telone (65%) and chloropicrin (35%) was used to fumigate beds before planting in October of each year.

Strawberry genotypes were selected based on availability and potential commercial value: Winterstar™, ‘Florida Radiance’, and ‘Strawberry Festival’ were commercially grown in Florida, while FL 06-38, FL 07-193, FL 09-127, and FL 10-47 were advanced selections from the University of Florida program. Four replicate plots (10 plants per plot) of each genotype were planted in a randomized complete block design in mid-October of 2011 and 2012. Fruit were hand harvested twice weekly when three-quarter red to fully red colored (standard commercial ripeness). From the four plots, a total of 20 strawberry fruit samples per genotype were harvested based on color and freedom of defects. Fruit was harvested on the mornings of 16 Jan. (mid-January), 30 Jan. (late-January), 13 Feb. (mid-February), and 27 Feb. (late-February) in 2012 and on 17 Jan. (mid-January), 31 Jan. (late-January), 14 Feb. (mid-February), and 28 Feb. (late-February) in 2013. From the 20 fruit per genotype harvested, 15 fruit were placed inside a clamshell (453 g; Wasserman Bag

Co., Inc., New York, NY) and the remaining five fruit were used for initial (0 d) chemical analysis. The clamshells containing the 15 fruit per genotype were placed on open racks in a refrigerated room (first year: 4.0 ± 0.5 °C and $90 \pm 2\%$ RH; second year 3.9 ± 0.3 °C and $89 \pm 3\%$ RH) for 7 d, to simulate commercial cold storage conditions. Note that due to the lower yield of some trial genotypes, in the harvest of mid-January of 2012 there were not enough fruit from genotypes FL 06-38, FL 09-127, and FL 10-47 available for evaluation at harvest and after storage. Therefore, fruit from these genotypes were only evaluated after storage. Similarly, in the harvest of mid-January of 2013 there were not enough fruit from genotype FL 06-38 available for evaluation at harvest and after storage. Therefore, fruit were only evaluated after storage.

Sample preparation. Strawberry fruit (5 fruit on day 0 and 15 fruit after 7 d of storage per genotype) were homogenized in a laboratory blender (Waring Products Div., Dynamics Corp. of America, New Hartford, CO) at high speed for 2 min. The homogenates for SSC, TA, and TP content were centrifuged at 12,000 rpm for 20 min at 4 °C and filtered through cheesecloth. Samples were frozen at –30 °C

until analysis, and once ready for analysis, were thawed overnight at 4 °C.

Weight loss and dry weight. Weight loss was calculated from the initial weight of 15 fruit per genotype and after 7 d using a precision electronic balance (Timberland Series TP-3102, Denver Instrument, New York, NY). Note that the concentrations of chemical constituents were expressed in terms of dry weight to show differences that might be obscured by differences in water content/loss. The following formula was used for water loss corrections: [chemical components (fresh weight) $\times$ 100 g $\div$ fruit dry weight + weight loss during storage (g)]. Dry weight was determined by drying a 5 g aliquot of homogenized fruit tissue in a laboratory oven at 80 °C until weight stabilized.

Soluble solids content. The homogenates (prepared as described above) were thawed and the SSC of the supernatant was determined by placing 0.3 mL of juice onto the prism surface of a digital ATAGO PR-101 refractometer in the 0% to 45% Brix range (Atago Co., Tokyo, Japan). The refractometer was calibrated using deionized water. SSC results are presented on a dry weight basis.

TS content. Frozen samples were thawed at 4 °C overnight, and three replicated samples of 2 g of fruit puree each were combined

Fig. 1. Weight loss of commercial strawberry cultivars and advanced selections obtained on four harvest dates during two consecutive years. (A) After 7 d at 4 °C for 2012; (B) after 7 d at 4 °C for 2013. Data are presented as means $\pm$ standard errors. Mean separations by the Fisher's least significant difference test at $P < 0.05$.

with 8 mL of ultrapure water ($\Omega 18-17$) and then centrifuged at 6000 rpm for 10 min. The obtained supernatant was filtered through a 0.45 μm nylon filter into 2 mL labeled vials. The high-performance liquid chromatography (HPLC) quantification of TSs was conducted using a Hitachi HPLC system with an refractive index detector and a 300 $\times$ 8 mm Shodex SP0810 column (Shodex, Colorado Springs, CO) with an SP-G guard column (2 $\times$ 4 mm). Isocratic solvent delivery of water was set at 1.0 mL $\cdot$ min $^{-1}$. Sample injection volume was 5 μL . Standard solutions of sucrose, glucose, and fructose (Fisher Scientific Company, Pittsburgh, PA) were used to identify sample peaks. After comparison of retention time with the standards, the peaks were identified. The amount of individual sugars in strawberry was quantified using calibration curves obtained from different concentrations (2, 4, 6, 10, and 20 mg $\cdot$ mL $^{-1}$) of sucrose, glucose, and fructose standards. Three samples per genotype (2 g fruit puree) were used, each with duplicate HPLC injections. TSs were calculated as the sum of the individual sugar values and expressed as g $\cdot$ kg $^{-1}$ on a dry weight basis.

Titrateable acidity. The supernatant of thawed homogenates was centrifuged at 12,000 rpm for 20 min. Three aliquots (6 g each) of the supernatant were diluted with 50 mL distilled water and titrated with 0.1 N NaOH to an endpoint of pH = 8.1 using an automatic titrator (Fisher Titrimeter II, No. 9-313-10, Pittsburgh, PA). The amount of NaOH (mL) required to reach the endpoint was recorded, and TA was calculated using the following equation: $\{[\text{vol. of NaOH (mL)} \times \text{normality} \times 0.064] \times 100\} / 6 \text{ g of juice}$ where 0.064 = milliequivalent factor for citric acid. The TA of strawberry was expressed on a dry weight basis.

Total AA. Total AA was quantified by mixing three replicated samples of 2 g of homogenate each with 20 mL metaphosphoric acid mixture (6% HPO $_3$ containing 2 N acetic acid). Samples were then filtered (0.22 μm) before HPLC analysis. The AA analysis was conducted using a Hitachi LaChromUltra UHPLC system with a diode array detector and a LaChromUltra C18 2 μm column (2 $\times$ 50 mm) (Hitachi Ltd., Tokyo, Japan). The analysis was performed under isocratic mode at a flow rate of 0.5 mL/min with a detection of 254 nm. Sample injection volume was 5 μL , each with duplicate HPLC injections. Mobile phase was buffered potassium phosphate monobasic (KH $_2$ PO $_4$, 0.5%, w/v) at pH 2.5 with metaphosphoric acid (HPO $_3$, 0.1%, w/v). The retention time of the AA peak was 2.5 min. After comparison of retention time with the AA standard, the peak was identified. The amount of total AA content in strawberry was quantified using calibration curves obtained from different concentrations (10, 20, 30, 50, 100, 150, 200, and 300 $\mu\text{g}\cdot$ mL $^{-1}$) of AA standards. The total AA content was expressed in g $\cdot$ kg $^{-1}$ on a dry weight basis.

TP content. Total soluble phenolic compounds were determined using the

Folin-Ciocalteu colorimetric method (Folin and Ciocalteu, 1927; Singleton and Rossi, 1965). Gallic acid was used as the standard, and the concentration of total soluble phenolics was expressed as g $\cdot$ kg $^{-1}$ on a dry weight basis.

TAC content. The homogenate (three replicated samples of 2 g each) was mixed with 28 mL of 0.5% (v/v) HCl in methanol and held for 1 h at 4 $^{\circ}\text{C}$ for pigment extraction. TAC content was measured using the method described by Spayd and Morris (1981). Results were expressed as g $\cdot$ kg $^{-1}$ on a dry weight basis.

Statistical analysis. Analyses of variance (ANOVA) were performed using general linear models with the year, harvest date, genotype, and storage as main effects and their interactions as fixed effects using SAS $^{\circ}$ software (version 9.3; SAS Institute Inc., Cary, NC). When the genotype $\times$ harvest date interactions were significant (F -test, $P \leq 0.05$), the data were analyzed separately by harvest date, within year. Significant differences between harvests and genotypes were detected using Fisher's protected least significant difference (LSD) test at $P \leq 0.05$.

Results and Discussion

Weight loss. Loss of moisture from strawberry is one of the major causes of overall

quality deterioration affecting not only the appearance of the fruit but also its compositional quality. Loss of weight is mostly caused by increased transpiration resulting in loss of water from the fruit to the environment. Water loss is strongly correlated with decrease in sugars, AA, TPs, and TAC contents in strawberry fruit (Nunes, 2015; Nunes and Emond, 2007; Nunes et al., 1998, 2005) because water loss disrupts membrane integrity causing cell leakage (Kissinger et al., 2005). Therefore, strawberry genotypes with higher resistance to loss of water would also be expected to show lower degradation in most of its chemical components during cold storage. In this study, strawberry weight loss was on average lower in 2012 compared with 2013 (4.9% and 7.3%, respectively) (Fig. 1A and B). The fact that fruit from the second year had, after 7 d, higher weight loss compared with fruit from the first year, may explain why the levels of all chemical components measured (SSC, acidity, AA, TPs, and TAC content) were also significantly lower in fruit from the second year (Figs. 2–7).

Overall, there was a significant ($P < 0.001$) variability due to the main effects (year, harvest date, genotype, and storage time) and their interactions on the weight loss of strawberry during storage, except for the harvest $\times$ genotype interaction which was

Fig. 2. Soluble solids content (SSC) of commercial strawberry cultivars and advanced selections obtained on four harvest dates during two consecutive years. (A) SSC at harvest for 2012; (B) SSC after 7 d at 4 $^{\circ}\text{C}$ for 2012; (C) SSC at harvest for 2013; and (D) SSC after 7 d at 4 $^{\circ}\text{C}$ for 2013. Data are presented as means $\pm$ standard errors. Mean separations by the Fisher's least significant difference test at $P < 0.05$.

Fig. 3. Total sugar (TS) content of commercial strawberry cultivars and advanced selections obtained on four harvest dates during two consecutive years. (A) TS at harvest for 2012; (B) TS after 7 d at 4 °C for 2012; (C) TS contents at harvest for 2013; and (D) TS after 7 d at 4 °C for 2013. Data are presented as means $\pm$ standard errors. Mean separations by the Fisher's least significant difference test at $P < 0.05$.

not significant ($P < 0.05$). There was no consistency among harvests for weight loss, particularly in the first year. For example, in the first year, weight loss was on average significantly higher in mid- and late-January than in mid- and late-February, regardless of the genotype (Fig. 1A). In the second year, weight loss was higher in fruit harvested in February compared with January, but the differences were considerably smaller (Fig. 1B). Since the storage conditions were almost identical during the 2 years, the differences in fruit weight loss between harvests were probably due to variability in the size of the fruit, the ratio between fruit surface area and mass/volume and calyx size. Differences in morphological characteristics between genotypes (e.g., thickness of the cuticle, and achene and stomata density) may also have contributed to differences in weight loss. Although no data were found in the literature regarding the relationship between the factors mentioned above and weight loss of strawberry during storage, in the present study results from linear regression analysis showed that there was a weak, yet significant, inverse correlation ($r = -0.12$; $P = 0.002$) between weight loss and fruit size, with smaller fruit having, in general, higher weight loss. The smaller fruit has higher surface area:volume ratios explaining somehow, the higher transpiration rates compared

with larger fruit (Díaz-Pérez, 1998; Lownds et al., 1993). In a previous study, Lownds et al. (1993) showed that postharvest water loss in pepper fruit was inversely related to fruit surface area. Díaz-Pérez (1998) conclude that size of eggplant fruit was an important factor in water loss and that the main route for eggplant water loss was the calyx. Thus, weight loss in eggplants declined with increase in fruit size because smaller fruit have higher surface area:volume ratios compared with larger fruit. Also, in small eggplant fruit with higher calyx:area ratios the amount of water lost through the calyx was significantly higher than in large fruit (Díaz-Pérez, 1998).

Regarding the variability in water loss between strawberry genotypes harvested in the first year, FL 07-193 and FL 09-127 had the highest weight loss (9.5% and 9.7%, respectively) in mid-January. In late-January, WinterstarTM had the highest weight loss (8.3%), and in mid- and late-February FL 07-193 had the highest weight loss (2.9% and 2.7%, respectively) (Fig. 1A). On average, 'Strawberry Festival' and 'Florida Radiance' had the lowest weight loss (4.2 and 4.5%, respectively) during the first year, whereas selection FL 07-193 had the highest (5.3%) In the second year, weight loss among genotypes appeared to be more consistent, with selection FL 07-193 having the highest weight loss from

late-January to late-February whereas 'Florida Radiance' had the lowest weight loss in late-January and late-February and selection FL 06-38 the lowest weight loss in mid-January and mid-February (Fig. 1B). On average, selection FL 07-193 had the highest weight loss (9.1%) and 'Strawberry Festival' the lowest weight loss (6.6%) of all genotypes harvested during 2013.

Soluble solids content. Overall, there was a significant ($P < 0.001$) variability due to the main effects (year, harvest date, genotype, and storage time) and their interactions with the SSC of strawberry. At harvest, there were significant differences among strawberry genotypes for SSC evaluated across the four harvest dates. In 2013, there was on average less variability in SSC between the different genotypes than in 2012 (Fig. 2A and C). However, fruit from the first year had on average significantly higher SSC content than that from the second year (95.8 and 88.9%, respectively). In mid-January of 2012, from the four genotypes evaluated, 'Strawberry Festival' and selection FL 07-193 had the highest SSC (98.0 and 97.2%, respectively) followed by WinterstarTM (95.7%) (Fig. 2A). From late-January to late-February 'Strawberry Festival' and selection FL 09-127 had the highest SSC compared with the other genotypes. Selection FL 09-127 harvested in late-January had the highest SSC (99.1%) among all strawberry cultivars and advanced selections on that date. On average, selections FL 07-193 and FL 09-127 and 'Strawberry Festival' had the highest SSC at harvest (96.1, 96.2 and 97.3%, respectively). Selection FL 07-193 and WinterstarTM showed relatively steady initial values of SSC from late-January to late-February (Fig. 2A). In 2013, selection FL 06-38 and 'Strawberry Festival' showed the highest SSC compared with the other genotypes; however, there was no significant difference between these and selections FL 09-127 and FL 10-47 (Fig. 2C). Overall, in both years, fruit harvested in January had on average higher SSC than fruit harvested in February. In a previous study, Pozo-Insfran et al. (2006) also reported that Florida strawberries harvested in January had higher SSC than fruit harvested in February. Similarly, Cayo et al. (2013) showed that commercial strawberry cultivars and advanced selections had, in general, higher SSC earlier than later in the season. For example, SSC of 'Strawberry Festival' declined from 8.3% in January to 6.1% in February and SSC of 'Florida Radiance' declined from 7.4% to 5.5% during the season (Cayo et al., 2013). The decline in SSC over the strawberry production year has been attributed to the higher field temperatures usually measured during late-February in Florida (Cayo et al., 2013; MacKenzie and Chandler, 2009).

After 7 d of storage at 4 °C, SSC significantly decreased regardless of the year, harvest or genotypes (Fig. 2B and D). However, a decrease in SSC content after storage was on average significantly higher in 2013 than in the 2012 (44.9% and 29.9%, respectively). On average, selection FL 09-127 showed the least

Fig. 4. Titratable acidity (TA) of commercial strawberry cultivars and advanced selections obtained on four harvest dates during two consecutive years. (A) TA at harvest for 2012; (B) TA after 7 d at 4 °C for 2012; (C) TA contents at harvest for 2013; and (D) TA after 7 d at 4 °C for 2013. Data are presented as means $\pm$ standard errors. Mean separations by the Fisher's least significant difference test at $P < 0.05$.

decrease and thus the highest SSC after storage across the two years (77.6% and 49.2%, respectively) compared with the other genotypes. On the other hand, SSC of 'Florida Radiance' was inconsistent, ranking lowest during most harvests during the first year (Fig. 1B) but ranking higher in late-January and late-February during the second year (Fig. 2D).

TS content. Overall, there was significant ($P < 0.001$) variability due to the main effects (year, harvest date, genotype, and storage time) and their interactions on the TS content of the strawberry genotypes. Unlike for SSC where, at harvest, fruit from the first year had on average a higher SSC than fruit from the second year (Fig. 2A and C), fruit from the first year had on average lower TS content than fruit from the second year (Fig. 3A and C). Also, the correlation between SSC and TS content was moderate ($r = 0.31$; $P = 0.020$) and not as high as previously reported by Whitaker et al. (2011). These differences may be explained by the fact that SSC measured by a refractometer may comprise other soluble components besides sugars, such as organic acids, AA, pigments, and phenolic compounds (Kader et al., 2003). In fact, acidity (Fig. 4A and C), TPs (Fig. 6A and C), and TACs (Fig. 7A and B) were on average higher in fruit from the first year which may explain the higher SSC but lower TS content in fruit from

the first year. Regardless of the low correlation between SSC and TS content found in this study, SSC was previously reported to correlate well with glucose and fructose content in strawberry and thus was suggested to serve as a good estimate of germplasm fructose and glucose contents (Whitaker et al., 2011).

Regarding genotype variations, in 2012, selections FL 09-127, FL 10-47, and 'Strawberry Festival' exhibited a decrease in TS content from late-January to late-February (Fig. 3A). However, in mid-February, selection FL 09-127 showed the highest TS content among all genotypes. Moreover, this selection showed only a slight decrease in TS content from late-January to late-February with values ranging from 708.0 to 796.0 g·kg⁻¹ which were among the highest values among all genotypes. On average, FL 09-127 had the higher TS content followed by FL 06-38 across all harvest dates. In 2013, there were considerable variations in TS content among genotypes at all harvest dates (Fig. 3C). Although FL 09-127 tended to have the highest TS content from late-January to late-February, it was not significantly different from other genotypes such as 'Strawberry Festival' and WinterstarTM (Fig. 3C).

TS content significantly decreased after storage regardless of the year, harvest date or genotype (Fig. 3B and D). However, there

was on average a significantly higher decrease in the TS content of fruit from the second year (44.5% decrease from harvest) compared with the first year (34.2% decrease from harvest). Selection FL 09-127 had the highest TS content after storage across the 2 years (an average of 543.5 and 635.5 g·kg⁻¹ for the first and second years, respectively). However, selection FL 06-38 had high TS content after storage in mid-January, late-January, and late-February, with values ranging from 453.0 to 732.0 g·kg⁻¹ during 2012 and, had on average similar TS content than selection FL 09-127 (Fig. 3B). While in the first year 'Florida Radiance' and WinterstarTM tended to have a greater variability in the direction of the change, in the second year, FL 07-193 had on average the lowest TS content among all genotypes (Fig. 3B and D).

Titratable acidity. Overall there was significant ($P < 0.001$) variability due to the main effects (year, harvest date, genotype, and storage time) and their interactions on TA. At harvest, TA was on average significantly higher in fruit from the first year compared with the second year (12.4% and 11.4%, respectively) (Fig. 4A and C). Although in the first year there was variability in TA, selection FL 10-47 and 'Strawberry Festival' had on average at the time of harvest the highest TA (14.7 and 14.2%, respectively) followed by 'Florida Radiance' (13.0%) (Fig. 4A). WinterstarTM and selections FL 06-38 and FL 09-127 had on average the lowest TA on the day of harvest (9.6, 11.7 and 11.3%, respectively). In the second year, selection FL 10-47 also had on average the highest TA (14.1%) compared with FL 09-127 and WinterstarTM, which had on average the lowest TA (9.7 and 8.7%, respectively) (Fig. 4C).

After storage, there was a significant difference ($P < 0.001$) among strawberry genotypes for TA evaluated across harvest dates during 2012 and 2013. Storage for 7 d at 4 °C resulted in a significant decline in TA of all genotypes, regardless of the year and harvest date (Fig. 4B and D). However, on average fruit from the first year showed a significantly lower decrease in TA after storage compared with fruit from the second year (30.4 and 53.7%, respectively). Selection FL 10-47 showed the highest TA after storage across the 2 years followed by 'Strawberry Festival'. Compared with the other strawberry genotypes, WinterstarTM had the lowest TA across the two years followed by selection FL 07-193. In a previous study, Cordenunsi et al. (2003) also reported a decline in TA in different strawberry cultivars stored for 6 d at 6 °C, particularly between harvest and the second day of cold storage.

Total AA. Overall, there was significant ($P < 0.001$) variability due to the main effects (year, harvest date, genotype, and storage time) and their interactions on the total AA content. Others have also reported significant variability in total AA content between different strawberry genotypes, years, and harvest dates (Singh et al., 2011; Tulipani et al., 2011). On average, AA content was higher in

Fig. 5. Total ascorbic acid (AA) content of commercial strawberry cultivars and advanced selections obtained on four harvest dates during two consecutive years. (A) AA at harvest for 2012; (B) AA after 7 d at 4 °C for 2012; (C) AA contents at harvest for 2013; and (D) AA after 7 d at 4 °C for 2013. Data are presented as means $\pm$ standard errors. Mean separations by the Fisher's least significant difference test at $P < 0.05$.

fruit from the second year compared with the first year except selections FL 06-38 and FL 07-193, which had significantly higher AA contents in the first year compared with the second year (Fig. 5A and C). On average, AA content of the different strawberry genotypes tended to decrease from mid-January to late-February, particularly in 2013. However, there was no consistency in AA content among the different strawberry genotypes between years. For example, in 2012, selection FL 07-193 had on average the highest AA content (7.5 g·kg⁻¹) followed by selection FL 06-38 and 'Strawberry Festival' (5.6 g·kg⁻¹) with WinterstarTM having the lowest AA content (3.8 g·kg⁻¹) among all genotypes (Fig. 5A). In 2013, 'Strawberry Festival', selection FL 10-47 and 'Florida Radiance' had on average the highest AA contents (6.7, 6.1 and 5.8 g·kg⁻¹, respectively) followed by selection FL 07-193 (5.7 g·kg⁻¹), with selection FL 06-38 having the lowest AA content (4.6 g·kg⁻¹) amongst all genotypes (Fig. 5C). Earlier, Stevens (1974) and Harris (1975) reported significant genotypic variation in the AA content of several fresh fruits and vegetables (FFVs). Several factors may contribute to the variability in AA content between different types of FFVs and amongst different cultivars within each FFVs category.

For example, Lee and Kader (2000) suggested that temperature and light intensity are the major preharvest contributors to the AA content of FFVs at harvest. Kader (1988) showed that AA content in FFVs is also significantly influenced by maturity at harvest and post-harvest handling conditions. Kalt (2005) suggested that the decline in AA content results from tissue degradation as the FFVs become overripe. In strawberries, loss of AA content is often enhanced by bruising due to cell wall damage and mobilization of enzymes such as ascorbate oxidase and ascorbate peroxidase, normally present in the cells and that once in contact with AA will cause its oxidation to dehydro AA (Klein, 1987; Nobile and Woodhill, 1981). Dehydro AA is highly unstable and is converted into 2, 3-diketo-L-gulonic acid, a compound which no longer has of vitamin activity (Davey et al., 2000).

Storage for 7 d at 4 °C resulted in a significant decrease in the AA content of all genotypes, regardless of the year and harvest date (Fig. 5B and D). Although AA content was higher for most of the genotypes harvested during the second year, a decrease in AA during storage was, in general, higher in fruit from the second year compared with the first year (37.2 vs. 26.5% decrease, respectively). In the first year, selection FL 07-193

had on average the highest AA content after storage followed by 'Strawberry Festival' (5.0 and 4.0 g·kg⁻¹, respectively). The least retention of AA after storage was observed for selection FL 10-47 and WinterstarTM (3.0 g·kg⁻¹). In the second year, however, selection FL 10-47 showed the lowest AA losses after storage followed by selection FL 07-193 (3.8 and 3.7 g·kg⁻¹, respectively). WinterstarTM was again the genotype with lowest AA retention after storage (3.1 g·kg⁻¹). Several other studies have also shown that in general AA content of FFVs significantly decreases during storage, but retention is greatly influenced by storage conditions, mostly by temperature. For example, Lee and Kader (2000) reported that AA content in fleshy fruits was generally lower after cold storage. Shin et al. (2007) noted a higher decrease in AA content when strawberry fruit were stored at 20 °C compared with 0.5 °C. In another study, Cordenunsi et al. (2003) reported a decrease of $\approx 50\%$ in the AA content of five strawberry cultivars when stored for 6 d at 6 °C. Nunes et al. (1998) also suggested that water loss in strawberry may intensify AA oxidation because fruit with higher water loss had, in general, the highest decrease in AA content. In this study, some genotypes such as 'Strawberry Festival', may have less proportional loss of AA due to a greater retention of water in the fruit during storage which was reflected by its relatively lower weight loss values (Fig. 1A and B) compared with the other genotypes. Cordenunsi et al. (2005) reported that lowering the storage temperature had different effects on levels of total AA over time, where the maintenance of initial AA values, less sensitivity to temperature changes, and a discrete diminution in AA were observed. The same authors reported that AA content fluctuations during storage seemed to be dependent on the type of cultivar, cultural practices, light intensity and climatic conditions. Certainly, factors such as preharvest climatic conditions and postharvest conditions influence the AA content of the fruit, but cultivar variations can be considered as an additional factor (Lee and Kader, 2000).

TP content. There was significant ($P < 0.001$) variability due to the main effects (year, harvest date, genotype, and storage time) and their interactions on the TP content of the strawberry fruit. In 2012, TP content was more variable between genotypes than in 2013 (Fig. 6C) which could be attributed to the fluctuation in average daily temperature throughout the 2012 production year, whereas in 2013, daily temperatures were more constant (Table 1). Previous studies have also reported the effects of environmental factors and genotype on TP content (Häkkinen and Törrönen, 2000; Rekika et al., 2005; Singh et al., 2011; Tulipani et al., 2011). On average, genotypes from the first year had significantly higher TP content compared with the second year (28.7 and 20.0 g·kg⁻¹, respectively) (Fig. 6A and C). In the first year, selection FL 07-193 had the highest TP content (31.6 g·kg⁻¹) followed by selection FL 10-47 and 'Strawberry Festival'

Fig. 6. Total phenolic content (TPC) of commercial strawberry cultivars and advanced selections obtained on four harvest dates during two consecutive years. (A) TPC at harvest for 2012; (B) TPC after 7 d at 4 °C for 2012; (C) TPC contents at harvest for 2013; and (D) TPC after 7 d at 4 °C for 2013. Data are presented as means $\pm$ standard errors. Mean separations by the Fisher's least significant difference test at $P < 0.05$.

(30.7 and 31.6 g·kg⁻¹, respectively) (Fig. 6A). WinterstarTM had on average the lowest TP content (22.9 g·kg⁻¹). In the second year, selections FL 10-47 and FL 06-38 had on average the highest TP contents (23.2 and 21.4 g·kg⁻¹, respectively), whereas FL 09-127 had the lowest TP content (17.2 g·kg⁻¹) (Fig. 6C).

Storage for 7 d at 4 °C contributed to a dramatically decreased TP content of all genotypes in both years (Fig. 6B and D). However, on average the decrease in TP content after storage was significantly higher in strawberry genotypes from the second year compared with the first year (29.3 and 50.4% decrease, respectively). Thus, it seems that higher levels of TP at harvest combined with lower weight loss during storage (Fig. 1) resulted in a higher TP retention after 7 d of storage. In the first year, selections FL 07-193, FL 10-47, and 'Strawberry Festival' retained their TP levels better than WinterstarTM, which showed the highest decrease in TP after storage (Fig. 6B). In the second year, selection FL 10-47 also had the highest TP retention, and WinterstarTM showed again the highest decrease in TP content after storage (Fig. 6D). Shin et al. (2007) reported a decline in TP content in strawberries stored at 0.5 and 10 °C on the second day of storage and a rapid

decline in fruit stored at 20 °C. Conversely, Ayala-Zavala et al. (2004) reported a gradual increase in TP in strawberries stored at 5 °C and 10 °C, whereas steady TP values were observed in strawberry fruit stored at 0 °C. However, the authors did not report the water loss of the fruit after storage which might have contributed to a concentration effect rather than to an actual increase in TP content. To determine the actual changes in TP content (as well as other chemical components), and to show the differences between temperatures that might be obscured by differences in water content, concentrations of chemical constituents should be expressed in terms of dry weight. In fact, Shin et al. (2008) reported that the marked decline in TP concentrations in ripe red fruit after 12 d of storage at 10 °C paralleled the increase in water loss and decreases in anthocyanin concentrations. Changes in TP contents may contribute not only to changes in the color of strawberries (darker, more red-brownish fruit) but may also contribute to changes in taste as some phenolic acids may have some astringent/bitter taste.

TAC content. Overall, there was considerable ($P < 0.001$) variability due to the main effects (year, harvest date, genotype, and storage time) and their interactions on the

TAC content of strawberry fruit. Several studies have previously shown that anthocyanin content varies significantly with the year, harvest date and genotype (Fredericks et al., 2012; Pozo-Insfran et al., 2006; Singh et al., 2011; Tulipani et al., 2011). The annual climatic changes during the season may result in different TAC accumulation patterns whereas specific genotype characteristic (e.g., surface color) are highly correlated with the TAC content of strawberries (Nunes, 2015). Also, storage temperature has a significant impact on the retention levels of TAC, as well as other polyphenolic compounds in the fruit (Cordenunsi et al., 2003; Jin et al., 2011).

In this study, TAC content was on average significantly higher in fruit from the first year compared with the second year (2.2 and 1.3 g·kg⁻¹, respectively), but significant variability among genotypes was observed (Fig. 7A and C). Within the first year, selection FL 10-47, 'Strawberry Festival' and Florida Radiance' had on average the highest TAC contents whereas selection FL 09-127 had the lowest (Fig. 7A). In general, TAC content increased from mid- to late-January, decreased in mid-February and then increased again in late-February. This trend may be correlated with field temperatures measured throughout the 2012 production year. In fact, temperatures increased from 13 in mid-January to 18 °C in late-January, decreased to 12 °C in mid-February and then increased to 18 °C in late-February (Table 1). So it seems that in the first year, the higher field temperatures may have contributed to an accumulation of TAC in the fruit. In the second year, however, there was an increase in TAC from mid-January to mid-February but then in late-February TAC content decreased. These difference might be related to less variation in field temperatures during the 2013 strawberry production year (Table 1). Nevertheless, the trend was somewhat similar to the TAC content of the different strawberry genotypes. That is, as in the first year, 'Florida Radiance' showed on average the highest TAC content (1.7 g·kg⁻¹) and selection FL 09-127 the lowest TAC content (0.8 g·kg⁻¹) (Fig. 7C). Since TAC content is highly correlated with strawberry surface color (Nunes, 2015) it can be deduced that fruit with higher TAC content would have a deeper red color compared with those with lower TAC content. Therefore, selection FL 09-127 which showed a lower TAC content would be lighter compared with 'Florida Radiance'. In fact, recently Whitaker et al. (2015) reported that the external and internal color of selection FL 09-127 is lighter than other cultivars.

After storage, TAC content significantly decreased regardless of the year, harvest date and genotype (Fig. 7B and D). However, the decrease in TAC content was on average significantly greater in fruit harvested in 2013 than in fruits from 2012 (21.9 and 41.6%, respectively). Thus, lower weight loss during storage (Fig. 1) combined with higher TAC content at harvest may have contributed to this positive retention. In the first year,

Fig. 7. Total anthocyanin content (TAC) of commercial strawberry cultivars and advanced selections obtained on eight harvest dates during two consecutive years. (A) TAC at harvest for 2012 year; (B) TAC after 7 d at 4 °C for 2012; (C) TAC contents at harvest for 2013; and (D) TAC after 7 d at 4 °C for 2013. Data are presented as means $\pm$ standard errors. Mean separations by the Fisher's least significant difference test at $P < 0.05$.

Table 1. Average, minimum, and maximum air temperatures collected in Balm, FL, during the 2012 and 2013 strawberry seasons (from Florida Automated Weather Network).

Yr	Harvest date	Temperature (°C)		
		Avg	Minimum	Maximum
2012	Jan. 16	14.0 ^a	5.0	22.0
	Jan. 30	16.0	9.0	24.0
	Feb. 13	10.0	0.0	20.0
	Feb. 27	23.0	18.0	29.0
2013	Jan. 17	16.0	13.0	22.0
	Jan. 31	14.0	6.0	21.0
	Feb. 14	16.0	15.0	18.0
	Feb. 28	16.0	10.0	22.0

^aAverage temperature from the day of harvest and the four previous days of each harvest date.

'Strawberry Festival' and 'Florida Radiance' had, after 7 d of storage, higher TAC levels (2.7 and 2.5 g·kg⁻¹, respectively) compared with selections FL 06-38 and FL 09-127 that showed on average lower TAC content (1.2 and 1.1 g·kg⁻¹, respectively) (Fig. 7B). In the second year, selection FL 10-47 better retained TAC after storage compared with selections FL 07-193, FL 09-127, and WinterstarTM, which had the least retention of TAC (Fig. 7D). These decreases are consistent with data reported by Ayala-Zabala et al. (2004) who found a significant decrease in

TAC content during 5 d of storage at 0 and 5 °C. López-Serrano and Ros Barceló (2001) reported that the instability of strawberry pigments and the effect of the oxidative enzyme polyphenol oxidase (PPO) on the degradation of anthocyanin, results in the loss of bright red color in strawberries. Nunes et al. (2005) also showed that physiological stress due to water loss during storage results in increased PPO activity and concluded that pigment degradation might be partly the result of an increase in PPO activity.

Impact of weather conditions on the composition of the different strawberry genotypes. According to the Florida Automated Weather Network (University of Florida IFAS, 2016), in mid-January, the average daily temperatures on the day of harvest were 13 and 19 °C in 2012 and 2013, respectively, whereas in late-February the average daily temperatures were 18 and 20 °C, respectively (Table 1). The irregularity of the temperature profiles might have contributed to AA, TP and TAC levels showing the same down-up pattern during the first year (Figs. 5A, 6A, and 7A). However, the influence of temperature was not as easily discernible in the second year, where AA, TP, and TAC decreased noticeably in the last harvest (Figs. 5C, 6C, and 7C) while temperature stayed relatively

steady. The high temperatures measured during late-February in 2012 (Table 1) most likely contributed to the decline in the SSC and TS content observed in most genotypes (Figs. 2A and 3A). Mackenzie and Chandler (2009) also reported a decline in SSC by the end of the strawberry production year in Florida, when temperatures were higher.

Strawberry genotypes also seemed to respond to field temperature differently. For example, selection FL 09-127 had the highest SSC and TS content on most of the dates (Figs. 2 and 3). This suggests that this genotype may have a consistently good eating quality which has been confirmed by taste panels (Whitaker et al., 2015). However, selection FL 09-127 had low AA, TP, and TAC contents compared with other genotypes (Figs. 5–7). Lower levels of TP and TAC were most likely associated with the genotype's lighter red color compared with other strawberry genotypes. 'Strawberry Festival' and selection FL 06-38 were the second highest genotypes for TS content throughout both years (Fig. 3). The behavior of WinterstarTM was noticeable because its TS content was more variable than that of other genotypes across harvest dates. Also its TA values were relatively low and constant. Similar results were observed in a previous study by Whitaker et al. (2012) who characterized WinterstarTM as a fruit with relatively low acid content—a trait that contributes to its mild, sweet flavor. Significant genotype $\times$ harvest date interactions ($P < 0.001$) for all sugar traits and TA demonstrated that advanced selections must be compared over multiple harvest dates to examine consistency and stability and to produce new cultivars with more consistent flavor. In this regard, breeding genotypes with higher and more stable SSC and sugar content toward the end of the year is a priority for the University of Florida strawberry breeding program.

Impact of cold storage on the composition of the different strawberry genotypes. Cold storage had a considerable effect on the levels of the aggregate constituents, showing clear trends to decrease over time for all compounds evaluated after 7 d of storage at 4 °C. Overall, development of genotypes that have both high and steady levels of sugars and moderate TA after storage is necessary. Selection FL 09-127 showed constantly high values of SSC and TS after storage (Figs. 2 and 3). Additionally, this selection usually had moderate TA somewhat similar to the commercial standard 'Florida Radiance' (Fig. 4B and C). Selection FL 10-47 had consistently high TA but had lower SSC and TS content in most evaluations. In contrast, selection FL 07-193 had lower ranges of values after storage for all these compositional traits.

Cold storage also had a significant impact on the levels of AA, TP, and TAC, which declined significantly after 7 d of storage (Figs. 5–7). Ultimately, genotypes that have both high and stable levels of these three compounds after storage are desirable.

Selection FL 07-193 exhibited consistently high values of AA and TP after storage but had consistently lower TAC (Figs. 5–7) compared with other genotypes. Selection FL 10-47 had lower AA levels in most evaluations but had consistently high TP and TAC contents. Conversely, Winterstar™ had lower values after storage for all traits. In contrast, ‘Strawberry Festival’ was somewhat variable for all classes of chemical compounds measured but was rarely the lowest for any given phytochemical after storage across harvest dates and years. Due to unpredictable weather fluctuations and significant interactions between genotype and harvest date for all compositional traits evaluated in this study, it is clear that genotypes must be compared over multiple years and several harvests within each year, to determine their performance compared with commercial cultivars and their stability during cold storage.

In conclusion, results from this study showed that genotype described a major portion of the variation in the chemical attributes of the fruit. However, chemical traits were also highly influenced by years, harvest dates within years and cold storage. Overall, selection FL 09-127 showed constantly high values of SSC and TS after storage, which is one of the factors that led to the release of this selection as Sensation® ‘Florida127’ (Whitaker et al., 2015). Moreover, FL 09-127 had a moderate TA content somewhat similar to the commercial standard ‘Florida Radiance’, whereas selection FL 10-47 had consistently high TA but in general had lower values of SSC and TS. A large effect of cold storage was perceived on AA, TP, and TAC contents resulting in a significant degradation of all chemical constituents. Steadily high values of AA and TP contents after storage were obtained for selection FL 07-193; however, TAC content was repeatedly lower compared with other genotypes. Conversely, selection FL 10-47 exhibited lower AA values but steadily high TAC and TPC contents. ‘Strawberry Festival’ was relatively variable for all classes of TS, TA, AA, TP, and TAC contents but was rarely the lowest for any given compound evaluated after storage across harvest dates and years. This moderate performance for all traits may have contributed to the commercial success of this cultivar and its reputation for consistent quality and excellent postharvest properties. Genotypes with greater yearly and harvest date stability for appearance, quality, and physicochemical traits after storage are desirable. Overall, results from this study provide valuable information to the selection process in breeding by identifying new genotypes with improved compositional attributes, combined with suitable quality characteristics after cold storage.

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