Anthocyanins and In Vitro Antioxidant Capacity of Strawberries from Different Disease Control Treatments

MARVIN ABOUTIOLAS AND M. CECILIA DO NASCIMENTO NUNES*

University of South Florida, Department of Cell Biology, Microbiology and Molecular Biology, Tampa, FL 33620

Additional index words: cold storage, weight loss, FRAP, DPPH, TEAC

Strawberry is one of the most appreciated fruits worldwide due to its delicate flavor. It is also an important source of bioactive compounds. However, in order to control pests and diseases, current agricultural practices involve almost weekly pesticide applications which may result in chemical contamination of the fruit and possible reduction of nutritional value. The objective of this work was to determine the effect of conventional, reduced-pesticide, or no-pesticide applications on anthocyanins, the major polyphenols in strawberry, and in vitro antioxidant capacity (AOC) of strawberries. ‘Strawberry Festival’ and ‘Florida Radiance’ strawberries from three different cultivation methods were harvested twice from commercial fields in Florida, stored at 1.5 °C and 85% relative humidity and evaluated daily during a seven-day storage period for total anthocyanin content and AOC. Results from this study showed that total anthocyanin and AOC of strawberries significantly decreased during storage, regardless of the cultivar and disease control treatment. Although at harvest, anthocyanin content and AOC was significantly higher in organic fruit, after storage the differences between organic and reduced-pesticide fruit were slight or nonsignificant. Overall, strawberries from the reduced-pesticide treatment showed better or similar anthocyanin content and AOC than fruit from the conventional disease control treatment. These results indicate that growing strawberries with reduced-fungicide applications can be an alternative to conventional disease control or organic practices as it reduces production costs and fungicide residues on the fruit while still retaining strawberry anthocyanins and AOC.

Strawberries are a valuable crop in Florida because they constitute an important source of revenue for the industry and are greatly appreciated by consumers due to their delicate flavor. Strawberries also have exceptional health benefits due to their high levels of bioactive compounds, including phenolic acids, flavonoids, and vitamin C. However, strawberry quality is greatly influenced by preharvest and postharvest conditions. In addition, strawberry growers in Central Florida face a major challenge when it comes to keeping fruit disease and fruit rot as low as possible. Anthracnose fruit rot, caused by Colletotrichum acutatum, and Botrytis fruit rot, caused by Botrytis cinerea, are the most common diseases of annual strawberries in Central Florida and worldwide (Pavan et al., 2011). Even in well-managed fields, losses from fruit rots can exceed 50% when conditions favor disease development (Ellis and Grove, 1982). For example, B. cinerea is the causal agent of Botrytis fruit rot, a major pre- and postharvest disease of strawberry (Sutton, 1998). The Eastern and especially the South-eastern United States appear to be highly conducive for fungicide resistance development in plant pathogenic fungi, which may be a result of the characteristic warm and humid climate enabling fungi to thrive and multiply. Therefore, in order to fight fruit rot and avoid considerable crop losses, strawberry growers need to apply protectant pesticides weekly through the season (Legard et al., 2001; Peres et al. 2010).

Well informed consumers however are gaining awareness of the health and environment hazards of extensive pesticide use thus alternative cultivation practices (i.e., organic), although more costly, are gaining popularity. Organic agricultural practices constitute a good alternative to conventional production because they exclude the use of synthetic fertilizers and pesticides, but require soil building and biological pest control. In addition, labor requirements may be as much as twice those of a conventional system (Pritts and Handley, 1999). An alternative approach to organic and conventional farming methods is the use of reduced pesticide applications based on a disease forecast system. Peres and MacKenzie (2009) showed that this system reduces the number of fungicide sprays by 50% without compromising disease control. By reducing the amount of fungicides used to control diseases through accurately targeting the right application time, overall postharvest quality of strawberry can be maintained, production costs reduced and an alternative to health-aware consumers can be provided.

Regardless of the preharvest treatments applied to the fruit, increased consumption of strawberries is considered a healthy choice. For example, strawberries have been acclaimed as a rich source of phytochemicals that include predominantly anthocyanins and phenolic acids with potential anti-carcinogenic and cardiovascular risk reduction properties (Hannum, 2004; Cerdá et al., 2005). These compounds are necessary for the growth and reproduction of strawberries, and also defend the plant against pathogens and...
environmental stress. In addition, anthocyanins are important components of strawberry because they contribute to the color and flavor of the fruit and seem to also be highly correlated with total antioxidant capacity (Wang and Lin, 2000). Since strawberries are fragile fruits, their quality and shelf life greatly depends on environmental conditions, mostly temperature, to which the fruit is exposed after harvest. Storage conditions may negatively affect the amount of bioactive compounds and antioxidant capacity and also result in excessive loss of water (Nunes and Dea, 2015). The objective of this work was to determine the effect of conventional, reduced-pesticide or no-pesticide applications on anthocyanins, the major polyphenols in strawberry, and on the in vitro antioxidant capacity of strawberries at harvest and during cold storage.

Material and Methods

PLANT MATERIAL AND FUNGICIDE TREATMENTS. ‘Florida Radiance’ and ‘Strawberry Festival’ strawberry cultivars were obtained from commercial fields in central Florida, and grown under the following disease management conditions: conventional, reduced-pesticide using a disease forecasting system (Peres and MacKenzie, 2009) and organic. ‘Florida Radiance’ and ‘Strawberry Festival’ strawberries grown conventionally or under a reduced-pesticide disease control treatment were harvested from commercial fields in Plant City and Floral City, respectively. ‘Strawberry Festival’ grown under organic conditions were obtained from a commercial field in Duette. ‘Florida Radiance’ strawberries grown under organic conditions were not available in Florida, therefore only organic ‘Strawberry Festival’ was tested against conventional and reduced-pesticide disease control treatments. The main commercial pesticides applied to the fruit were: Captan (captan), Captec (captan), Quilt Xcel (propiconazole + azoxystrobin), Thiram (thiram), Switch (cyprodinil + fludioxonil), Elevate (fenhexamid), Torino (cyllufenamid) and Fontels (penthiopryrad). For conventionally grown strawberries, single or combinations of different pesticides were applied early in the season, during the bloom and late in the season, with up to 24 applications during the season. For reduced-pesticide fruit, fungicides were applied only when environmental conditions were favorable for disease. That is, the number of pesticide applications was reduced by 50% or more. Organic strawberries were grown according the USDA National Organic Program (NOP) guidelines (USDA, 2014).

POSTHARVEST TREATMENTS. Strawberries from each cultivar and disease control treatment were harvested twice during the 2014 strawberry production season: ‘Florida Radiance’ from the two different disease control treatments (conventional and reduced-pesticide) were harvested on 21 Jan. (Harvest 1) and on 18 Feb. (Harvest 2) and ‘Strawberry Festival’ from the three different disease control treatments (conventional, reduced-pesticide and organic) were harvested on 7 Feb. (Harvest 1) and 7 Mar. (Harvest 2). Fruit were brought to the Food Quality Laboratory at the University of South Florida in Tampa with minimal delay after harvest (30 min to 1 h, depending on the location of the field). Upon arrival to the laboratory, fruit were selected for uniformity of size, color and lack of defects. They were carefully packed into 0.453 kg-clamshells (Wasserman Bag Co., Inc, Holbrook, NY) and stored at 1.5 °C and 85% relative humidity (RH) inside temperature and RH-controlled chambers (Forma Environmental Chambers Model 3940 Series, Thermo Electron Corp., Marietta, OH). These conditions simulated the lowest temperature and highest RH measured during strawberry handling (Nunes et al., 2009; Lai et al., 2011; Pelletier et al., 2011). Strawberry anthocyanin content and antioxidant capacity were evaluated at harvest and daily during a seven-day storage period.

TEMPERATURE AND RELATIVE HUMIDITY MONITORING. Temperature was monitored throughout the study using Stow Away™XTI02 temperature loggers (−5 °C to +37 °C) (Onset Computer Corporation, Pocasset, MA). RH was monitored with Stow Away™RH loggers (10 to 95% RH) (Onset Computer Corporation, Pocasset, MA).

WEIGHT LOSS AND DRY WEIGHT. Weight loss of three replicated samples of 15 strawberries each was calculated from the initial weight of the fruit and daily during a seven-day storage period. Concentrations of chemical constituents were expressed in terms of dry weight in order to show the differences between cultivars that might be obscured by differences in water content. The following formula was used for water loss corrections: [chemical components (fresh weight) x 100 g/strawberry dry weight + weight loss during storage (g)]. Strawberry dry weight was determined by drying three weighed aliquots of homogenized strawberry tissue at 80 °C, and until weight stabilized.

TOTAL ANTHOCYANIN CONTENT. Anthocyanins were extracted in 0.5% (v/v) HCl in methanol and measured using the procedure described by Nunes et al. (2005). The amount of total anthocyanin content was expressed in mg/g on a dry weight basis.

ANTIOXIDANT ASSAYS. The ferric ion reducing antioxidant power (FRAP) assay was conducted according to the method of Benzie and Strain (1996) with some modifications. The oxidant was prepared by mixing 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ) (2.5 mL of 10 mM TPTZ in 40 mM HCl), 20 mM Ferric chloride solution and 25 mL of acetate buffer (pH 3.6) in a 1:1:10 ratios, respectively. The resulting reagent was stable for at least three hours at room temperature. The FRAP reagent (160 µL) was mixed with 40 µL of sample in a 96 - well plate and then incubated at 37 °C for 30 min before measuring the absorbance at 593 nm.

The 2,2-diphenyl-1-picyrylhydrazyl radical scavenging capacity (DPPH) assay was conducted according to the method of Brand-Williams et al. (1995) with some modifications. The oxidant in the DPPH assay was prepared by mixing 3.5 mL of DPPH stock solution, consisting of 20 mg of DPPH in 100 mL methanol, with 6.5 mL of methanol prior to the procedure. The DPPH reagent (950 µL) was mixed with 50 µL of sample and then incubated at room temperature for 1 h in the dark. The absorbance of 200 µL of the mixture was read at 515 nm.

The Trolox equivalent antioxidant capacity (TEAC) assay was conducted according to the method of Arts et al. (2004) with some modifications. The oxidant was prepared by mixing 7.4 mM [2,2’-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)] ABTS with 2.6 mM potassium persulfate. The two solutions were allowed to react for at least 12 hours at room temperature in the dark to create the ABTS+ reagent. The resulting solution was diluted by mixing 1 mL of ABTS+ with 10 mL of methanol to obtain an absorbance of 1.5 units at 734 nm. The diluted reagent (980 µL) was mixed with 20 µL of sample and the absorbance of 200 µL of the resulting mixture was read at 734 nm.

For all three assays, results were calculated using a calibration curve of 20 mM Trolox standard in methanol and were expressed as micromoles of Trolox equivalents per 100 mL of sample (µmol TE/100 mL). All assays were run twice with triplicate samples each time. Stock solution of Trolox was prepared to a concentration of 20 mM in methanol and stored at −20 °C. From this stock solution, standards were freshly prepared prior to each assay in

concentrations of 1000 μM to 200 μM (FRAP and DPPH) and 3000 μM to 600 μM (TEAC). Absorbance of the samples and standards was read using a microplate reader (Biotek Instruments, Inc., Highland Park, VT). Antioxidant capacity was expressed in μmol·g⁻¹ of Trolox on a dry weight basis.

**Statistical Analysis.** The Statistical Analysis System computer package (SAS Institute, Inc., 2004) was used for the analysis of the data from these experiments. The two-way analysis of variance (ANOVA) was used with harvest, cultivar and disease control treatment as main effects. Significant differences between cultivars and disease control treatments were detected using the least significant difference (LSD) at the 5% level of significance.

**Results and Discussion**

**Weight loss.** Weight loss is an important factor when evaluating postharvest strawberry quality because excessive loss of moisture results in accelerated quality deterioration and loss of economic value (Nunes and Emond, 2007). Most weight loss of stored fruit is caused by transpiration, which can be controlled by raising the relative humidity (RH) of the surrounding environment. When RH falls below 80%, strawberry fruit tends to lose more water which may lead to cell wall damage and increased degradation of anthocyanins and other soluble phenolic compounds. Nunes et al. (2005) showed that in strawberries, greater water loss was associated with lower concentrations of anthocyanins and other soluble phenolic compounds, and higher polyphenol oxidase (PPO) activity (Nunes et al., 2005). Since bioactive compounds such as ascorbic acid and some phytochemicals are water soluble, when strawberry weight loss increases above a certain level (5% or more), the amounts of these compounds significantly decreases (Nunes et al., 1998; Nunes and Dea, 2015). The increase in PPO activity in strawberries that had a significant loss of water during storage was associated with the decrease in anthocyanins and to fewer dark red fruit (Nunes et al., 2005). In this study, there was a significant loss in weight after seven days of storage for both ‘Strawberry Festival’ and ‘Florida Radiance’ (Fig. 1). On average, ‘Strawberry Festival’ had greater weight loss compared to ‘Florida Radiance’ strawberries, regardless of the harvest or the disease control treatment used. At the first harvest, ‘Florida Radiance’ from conventional and reduced pesticide control treatments showed an average of 6% weight loss after seven days of storage, with no significant difference between treatments. On the other hand, after seven days of storage there was less weight loss for ‘Strawberry Festival’ in conventional fruit compared to fruit from the reduced pesticide treatment. Organic ‘Strawberry Festival’ had significantly less weight loss (7.9%) compared to conventional ‘Strawberry Festival’ (9.4%) and reduced pesticide ‘Strawberry Festival’ (10.2%). In the second harvest, there was a less marked difference in weight loss of fruit from the different treatments (Fig. 1). Weight loss of ‘Florida Radiance’ from the conventional treatment was significantly greater than that of fruit from reduced pesticide treatment (9.1% and 7.7%, respectively). ‘Strawberry Festival’ from the reduced pesticide treatment showed the least weight loss (7.5%) compared to the conventional and organic treatments (8.9% and 9.1%, respectively). On average, fruit from the reduced pesticide treatment tended to have similar or less weight loss than that of strawberry from the conventional disease control treatment. Differences in weight loss between cultivars and disease control treatments may be a result of variations in the morphological characteristics of the fruit. For example, the thickness of the skin may determine the amount of moisture lost during storage. That is, the thicker the skin of the fruit, the lower the amount of lost moisture and thus the lower the amount of weight loss (Nunes and Emond, 2007).

**Total Anthocyanins.** Overall, anthocyanin content decreased during cold storage regardless of the cultivar, disease control treatment or date of harvest (Fig. 2). Although, fruit from the first harvest had, in general, lower anthocyanin content at harvest (day 0) compared to fruit from the second harvest, the decrease in anthocyanin content was greater for the later harvest. At the first harvest, total anthocyanin content fluctuated from day to day across all cultivars and disease control treatments (Fig. 2). At harvest (day 0), the strawberry cultivars had different total anthocyanin content ranging from a minimum of 88.7 mg/100 g in conventional ‘Strawberry Festival’ to a maximum of 157.8 mg/100 g in conventional ‘Florida Radiance’ strawberry. After seven days of storage, there was a significant decrease in anthocyanin content in fruit from both the conventional and reduced pesticide treatments for both cultivars. The anthocyanin content of organic ‘Strawberry Festival’ remained relatively stable throughout storage showing the smallest decrease (6.4%) after seven days, compared to fruit from the conventional (22.9%) and reduced pesticide treatments (49.5%). Even though at harvest fruit from the reduced pesticide treatment had higher anthocyanin content (123.5 mg/100 g) than fruit from the conventional treatment (88.7 mg/100 g), the amounts of these compounds significantly decreases when strawberry weight loss increases above a certain level (5% or more), the amounts of these compounds significantly decreases (Nunes et al., 1998; Nunes and Dea, 2015). The increase in PPO activity in strawberries that had a significant loss of water during storage was associated with the decrease in anthocyanins and to fewer dark red fruit (Nunes et al., 2005). In this study, there was a significant loss in weight after seven days of storage for both ‘Strawberry Festival’ and ‘Florida Radiance’ (Fig. 1). On average, ‘Strawberry Festival’ had greater weight loss compared to ‘Florida Radiance’ strawberries, regardless of the harvest or the disease control treatment used. At the first harvest, ‘Florida Radiance’ from conventional and reduced pesticide control treatments showed an average of 6% weight loss after seven days of storage, with no significant difference between treatments. On the other hand, after seven days of storage there was less weight loss for ‘Strawberry Festival’ in conventional fruit compared to fruit from the reduced pesticide treatment. Organic ‘Strawberry Festival’ had significantly less weight loss (7.9%) compared to conventional ‘Strawberry Festival’ (9.4%) and reduced pesticide ‘Strawberry Festival’ (10.2%). In the second harvest, there was a less marked difference in weight loss of fruit from the different treatments (Fig. 1). Weight loss of ‘Florida Radiance’ from the conventional treatment was significantly greater than that of fruit from reduced pesticide treatment (9.1% and 7.7%, respectively). ‘Strawberry Festival’ from the reduced pesticide treatment showed the least weight loss (7.5%) compared to the conventional and organic treatments (8.9% and 9.1%, respectively). On average, fruit from the reduced pesticide treatment tended to have similar or less weight loss than that of strawberry from the conventional disease control treatment. Differences in weight loss between cultivars and disease control treatments may be a result of variations in the morphological characteristics of the fruit. For example, the thickness of the skin may determine the amount of moisture lost during storage. That is, the thicker the skin of the fruit, the lower the amount of lost moisture and thus the lower the amount of weight loss (Nunes and Emond, 2007).

![Fig. 1. Changes in weight loss of ‘Strawberry Festival’ and ‘Florida Radiance’ strawberries from different disease control treatments during storage at 1.5 °C and 85% RH. F = ‘Strawberry Festival’, R = ‘Florida Radiance’.](image-url)

![Fig. 2. Changes in total anthocyanin contents of ‘Strawberry Festival’ and ‘Florida Radiance’ strawberries from different disease control treatments during storage at 1.5 °C and 85% RH. F = ‘Strawberry Festival’, R = ‘Florida Radiance’.](image-url)
mg/100 g), the decrease during cold storage was significantly greater for reduced pesticide fruit vs. conventional fruit (49.5% and 22.9%, respectively). For ‘Florida Radiance’, the decrease in the anthocyanin content in fruit from the conventional treatment was greater (35.9%) compared to the decrease in fruit from the reduced pesticide treatment (27.2%). Although fruit from the reduced pesticide treatment at harvest had a lower anthocyanin content (147.7 mg/100 g) compared to fruit from the conventional treatment (157.81 mg/100 g), the decrease during cold storage was significantly greater in conventional fruit. For the second harvest, anthocyanin content also fluctuated throughout storage with a significant decrease after seven days of storage, regardless of the cultivar or disease control treatment (Fig. 2). At harvest (day 0), total anthocyanin content ranged from a minimum of 148.6 mg/100 g in reduced pesticide ‘Florida Radiance’ strawberries to a maximum of 249.7 mg/100 g in organic ‘Strawberry Festival’, which is in agreement with previously published data (Crecente-Campo et al., 2012). Even though Reganold, et al. (2010) showed that anthocyanin content was higher in organic strawberries compared to conventionally grown fruit, in the present study, organic ‘Strawberry Festival’ from the first harvest had significantly lower anthocyanin content than conventional ‘Florida Radiance’ (132.44 and 157.81 mg/100 g, respectively). Although organic fruit tended to have higher anthocyanin content at harvest, they showed a significant decrease (56.3%) during storage when compared to fruit from the conventional (60.0%) and reduced (34.7%) treatments. ‘Florida Radiance’ from the conventional treatment had a smaller decrease (38.4%) in anthocyanin content during storage compared to reduced pesticide ‘Florida Radiance’ strawberry (48.8%). Fruit from the reduced pesticide treatment tended to have similar or significantly higher anthocyanin content compared to the conventional fruit whereas organic fruit had consistently higher anthocyanin content compared to the other treatments after seven days in cold storage (Fig. 2). Overall, losses in anthocyanin content ranged from 6.4% for organic ‘Strawberry Festival’ from the first harvest to 60.0% for conventional ‘Strawberry Festival’ from the second harvest. Therefore, the conventional disease control treatment did not seem to offer better protection to the fruit in terms of anthocyanin retention than the reduced pesticide or organic growing methods. In addition, weight loss and anthocyanin degradation after cold storage was significant, regardless of the cultivar or disease control treatment, suggesting that water soluble anthocyanins can easily be lost even with optimal storage conditions.

**Antioxidant Capacity.** Overall, the antioxidant capacity (AOC) of strawberries significantly decreased during storage, regardless of the cultivar, disease control treatment or date of harvest (Fig. 3). In addition, the different AOC assays used showed similar decreasing trends, regardless of the cultivar, disease control treatment or date of harvest. For the first harvest, there was, on average, a 57.9% decrease in the AOC of conventional ‘Strawberry Festival’, followed by a 56.3% and 52.1% decrease in fruit from the conventional and organic pesticide treatments, respectively. Organic ‘Strawberry Festival’ showed consistently higher FRAP, DPPH and TEAC values when compared to the other treatments (Fig. 3). These results are supported by previous published data, where TEAC values were also higher in organic strawberries when compared to conventional fruit (Reganold et al., 2010). In general, there was no significant difference between the AOC of conventional and reduced pesticide ‘Strawberry Festival’. Similarly, conventional ‘Florida Radiance’ showed a greater decrease in AOC after cold storage (46.8%) when compared to fruit from the reduced pesticide treatment (40.7%). Overall, there was no significant difference in the AOC of conventional and reduced pesticide ‘Florida Radiance’. For the second harvest, there was a similar trend, with organic ‘Strawberry Festival’ showing consistently higher FRAP, DPPH and TEAC values when compared to the conventional or reduced pesticide fruit (Fig. 3). In addition, ‘Strawberry Festival’ from the organic treatment showed the least decrease in AOC throughout storage (52.9%) when compared to conventional (55.4%) and reduced pesticide fruit (56.1%). These results however do not support the significant decrease observed in the anthocyanin content in organic ‘Strawberry Festival’, indicating that anthocyanins may not be the only phytochemical compounds responsible for AOC in strawberries, as previously suggested by Ha and To (2000). ‘Strawberry Festival’ from the reduced pesticide treatment had similar or slightly lower FRAP, DPPH, and TEAC values than conventional fruit. Although ‘Florida Radiance’ strawberries from the conventional treatment had higher FRAP, DPPH, and TEAC values at harvest (days 0), they showed a smaller decrease in AOC after seven days of cold storage compared to the reduced pesticide fruit (57.1 and 42.2%, respectively). There was no significant difference in FRAP, DPPH, and TEAC values between ‘Florida Radiance’ strawberries from the conventional or reduced pesticide treatments (Fig. 3).
Previous studies reported that total anthocyanins and AOC vary significantly among growing environments (Wang and Lin, 2003; Wang and Zheng, 2001) but during cold storage strawberry fruit from conventional, reduced pesticide or organic cultivation methods all had a similar decreases in anthocyanins and AOC. Overall, after seven days of cold storage FRAP, DPPH, and TEAC values were reduced by approximately 50%. However, the decrease in AOC was not significantly correlated with the decrease in total anthocyanin content, probably because anthocyanin content fluctuated from day to day and did not consistently decrease throughout cold storage. These results further suggest that, even though anthocyanins make up the majority of the polyphenol content in strawberries, other compounds such as vitamin C and phenolic acids may significantly contribute to overall AOC of the fruit (Jin et al., 2011).

Conclusions

Organic ‘Strawberry Festival’ strawberries showed consistently higher anthocyanin content and AOC compared to conventional or reduced pesticide fruit. ’Florida Radiance’ strawberries from the reduced pesticide treatment had greater or similar anthocyanin content and AOC compared to the conventional disease control treatment. These results indicate that growing strawberries with reduced fungicide applications can be an alternative to conventional disease control treatments or organic practices as it may help reduce production costs and fungicide residues on the fruit while still retaining strawberry anthocyanins and the antioxidant capacity of the fruit.

Literature Cited


