Journal of the American Pomological Society 79(1): 1-6 2025. https://doi.org/10.71318/r4s8ab07.

Anthocyanin Content and Profiles of Strawberry Fruit from North Carolina Genotypes

BRIANNA HAYNES[1,2], GINA FERNANDEZ[1], GUOYING MA[1,2], PENELOPE PERKINS-VEAZIE[1,2,3]

Additional index words: flavonoids, *Fragaria ×ananassa*, fruit color, pelargonidin-3-O-glucoside, pelargonidin-3-O-rutinoside

Abstract

Anthocyanins impart the pink to orange-red to deep red color found in strawberry (*Fragaria ×ananassa*) fruit. In this study, the anthocyanin content and profile of advanced North Carolina selections were compared to those of white to moderately-red fruited commercial cultivars. Pelargonidin-3-O-glucoside (P3G), pelargonidin-3-O-rutinoside (P3R), pelargonidin-3-O-(6"-malonylglucoside) (P3MG), and cyanidin-3-O-glucoside (C3G) were identified in strawberry fruit juice using high-performance liquid chromatography and the sum of these values represented total anthocyanin content (TAC). The dominant pigment, P3G, ranged from 0.2 to 69.2 mg/100g in white and red fruit, respectively, and values represented 60 to 89% of TAC. Across genotypes, the anthocyanins P3R and C3G represented 4 to 16% and 1 to 17%, respectively, of TAC. Generally regarded as a minor pigment, P3MG contributed 9–18% TAC in nine North Carolina genotypes and 9-10% TAC in cultivars 'Sweet Charlie' and 'Ruby June'. Overall, North Carolina genotypes had anthocyanin content and profiles similar to the commercial cultivars 'Camarosa', 'Chandler', 'Sweet Charlie', 'Felicity', 'Medallion', and 'Ruby June'. Visual strawberry fruit color appears to be related more to the total amount of anthocyanin than to specific anthocyanin profiles.

Introduction

Strawberry (*Fragaria* × *ananassa*) fruit color ranges from white to dark purple-red (Fig. 1). Flavonoid anthocyanins, composed of glycosylated anthocyanidins, provide most of the strawberry visual color (Aaby et al. 2012). Most strawberry anthocyanin is composed of pelargonidin, contributing an orange to bright red color, while the small amounts of cyanidin present can impart a darker red to magenta color (Chaves-Silva et al. 2018). Pelargonidin-3-O-glucoside (P3G), pelargonidin-3-O-rutinoside (P3R), and cyanidin-3-O-glucoside (C3G) have been identified in red-fruited strawberries, although relative amounts can differ widely across genotypes (55–95% for P3G, 0–17% for P3R, and 1–8.9% for C3G) (Dzhanfezova et al. 2020).

The North Carolina (NC) strawberry breeding program provides material that is suitable for plasticulture production across the relatively diverse growing conditions of the South Atlantic US region. Fully ripe and

[1]North Carolina State University, Department of Horticultural Science, 2721 Founders Drive, Raleigh, North Carolina 27607
[2]North Carolina State University, Plants for Human Health Institute, 600 Laureate Way, Kannapolis, North Carolina 28081
[3]This research was supported in part by NIFA USDA Hatch 02977
[3]Corresponding author Penelope_perkins@ncsu.edu

red strawberries are generally preferred for this area, where local and direct sales make up the majority of the market (Samtani et al. 2019). Although the North Carolina breeding program has been active since the early 1900s, the anthocyanin content and profile of NC strawberries have been published for only a few genotypes (Perkins-Veazie et al. 2016). The objective of this study was to determine if the fruit anthocyanin profile of advanced North Carolina selections differed from that of commercial cultivars of white to dark red colors.

Fig. 1. Color of strawberry fruit juice extracted from germplasm grown in North Carolina **(A)**, fruit in clamshells of 'Florida Pearl', 'Sweet Charlie', 'Felicity', NC21-031, and NC19-020 (left to right) **(B)**, and singular fruits from the genotypes **(C)**.

Materials and Methods

Octoploid strawberry fruit from 13 genotypes from the North Carolina breeding program was compared with that from two classic strawberry cultivars ('Chandler' and 'Camarosa'), a strawberry cultivar ('Ruby June') widely grown in NC, and four cultivars commonly grown in Florida ('Felicity', 'Medallion', 'Sweet Charlie', and 'Florida Pearl'). All genotypes were planted as a field trial at the Piedmont Research Station in Salisbury, NC (35.69501°, -80.62939°) in September of 2022 using a plasticulture system (Hoffmann et al. 2024). The trial was arranged as a randomized complete block (RCB), with 3 blocks of 10 plants per block per genotype. Additional details on these field plantings can be found in Haynes (2024).

Weather challenges during the 2023 harvest season prevented incorporation of the RCB for fruit collection for anthocyanins. Instead, fully ripe berries were harvested at three weekly harvest dates per genotype from April through May of 2023, and samples consisted of five fruits pooled across blocks for each harvest date. Fruit were subjectively evaluated for visual color using standardized RosBREED phenotyping protocols (RosBREED 2024), where 1 = white; 9 = very red. Fruit were frozen and held at -80ºC until analysis. Each 5-fruit sample was thawed at room temperature, and juice extracted with acidified methanol to determine total anthocyanin content (TAC) and pigment profiles using a high-performance liquid chromatograph (Hitachi LaChrom, Tokoyo, Japan) following the methods of Perkins-Veazie et al. (2016). Anthocyanin concentrations were determined using standard curves from pelargonidin-3-O-glucoside (P3G), and individual anthocyanins were identified by retention times compared to external standards of P3G, P3R, C3G (Chromadex, Irvine, CA, USA; Sigma, St. Louis, MO, USA) and previous reports for P3MG (Fredericks et al. 2012; Lopes da Silva et al. 2007). Total anthocyanin content

https://doi.org/10.71318/r4s8ab07

represents to sum of the identified anthocyanins as mg P3G equivalents/100 g fresh wt. The relative content of anthocyanin pigments [100% x ({mg/100 g individual pigment}/{mg/100g total anthocyanin content})] was also measured. The laboratory study was designed as a completely randomized design, with replicates consisting of the three harvest dates. Data were subjected to JMP (version 15.1, SAS Institute, Cary, NC, USA), and means separated using Tukey's Honestly Significant Difference (HSD).

Results and Discussion

In this study, the TAC of strawberry juice ranged from 0.2 mg/100 g in the white 'Florida Pearl' to 69.2 mg/100 g in the moderately or dark red genotypes such as NC21-033 and NC19-020 (Fig. 2A). The relative content of the four anthocyanins varied among the germplasm. Pelargonidin-3-O-glucoside (P3G) represented 60 to 89% of TAC and was not significantly different among genotypes (data not shown). The percent pelargonidin-3-O-rutinoside (P3R) varied widely among genotypes, from 4 to 17% (Fig. 2B). Pelargonidin-3-O-(6"-malonylglucoside) (P3MG), which is not always detected in strawberry cultivars (Davik et al. 2020), was relatively high (9–18%) in five of the NC selections and the NC cultivars 'Rocco' and 'Liz' (Fig. 2C). Cyanidin-3-O-glucoside (C3G) has a darker red color than the orange-red pelargonidin (Dzhanfezova et al. 2020). Only small amounts of C3G were detected in the strawberry genotypes in this study, with relative content higher in the light-colored cultivars 'Florida Pearl', 'Ruby June', and 'Liz' than in the moderately red genotypes (Fig. 2D). Anthocyanin metabolism differs among germplasm, and the concentrations of C3G and P3G, as well as fruit color, have been linked to the expression levels of the *FaMYB10* transcription factor and *FaF3'H* gene (Lin et al. 2018). 'Florida Pearl', which can be white with a small amount of red color (often on the achenes) (Whitaker et al. 2023), was found to contain the same four pigments as the moderately red strawberries.

Values for 'Camarosa', 'Chandler', 'Liz', and 'Rocco' are similar to those reported by others (Fredericks et al. 2012; Perkins-Veazie et al. 2016; Vinson et al. 2022). In the current study, the total and relative anthocyanin content had more variation with harvest date than expected, as indicated by standard deviations (Fig. 2). Similar variation in strawberry anthocyanin has been noted for year, harvest date, and genotype by others, indicating that multiple seasons of data are desirable for determining anthocyanin content (Cayo et al. 2016; Fredericks et al. 2012; Tulipani et al. 2011).

Conclusions

In this study, total and individual anthocyanins of juice from strawberry germplasm of white to dark red fruit color were determined and varied widely depending on genotype. North Carolina strawberry genotypes were found to be similar in anthocyanins to those from commercial cultivars. These findings indicate that strawberry fruit visual color (white to dark red) appears to be dependent on the total amount of anthocyanin rather than individual pigment contribution.

https://doi.org/10.71318/r4s8ab07

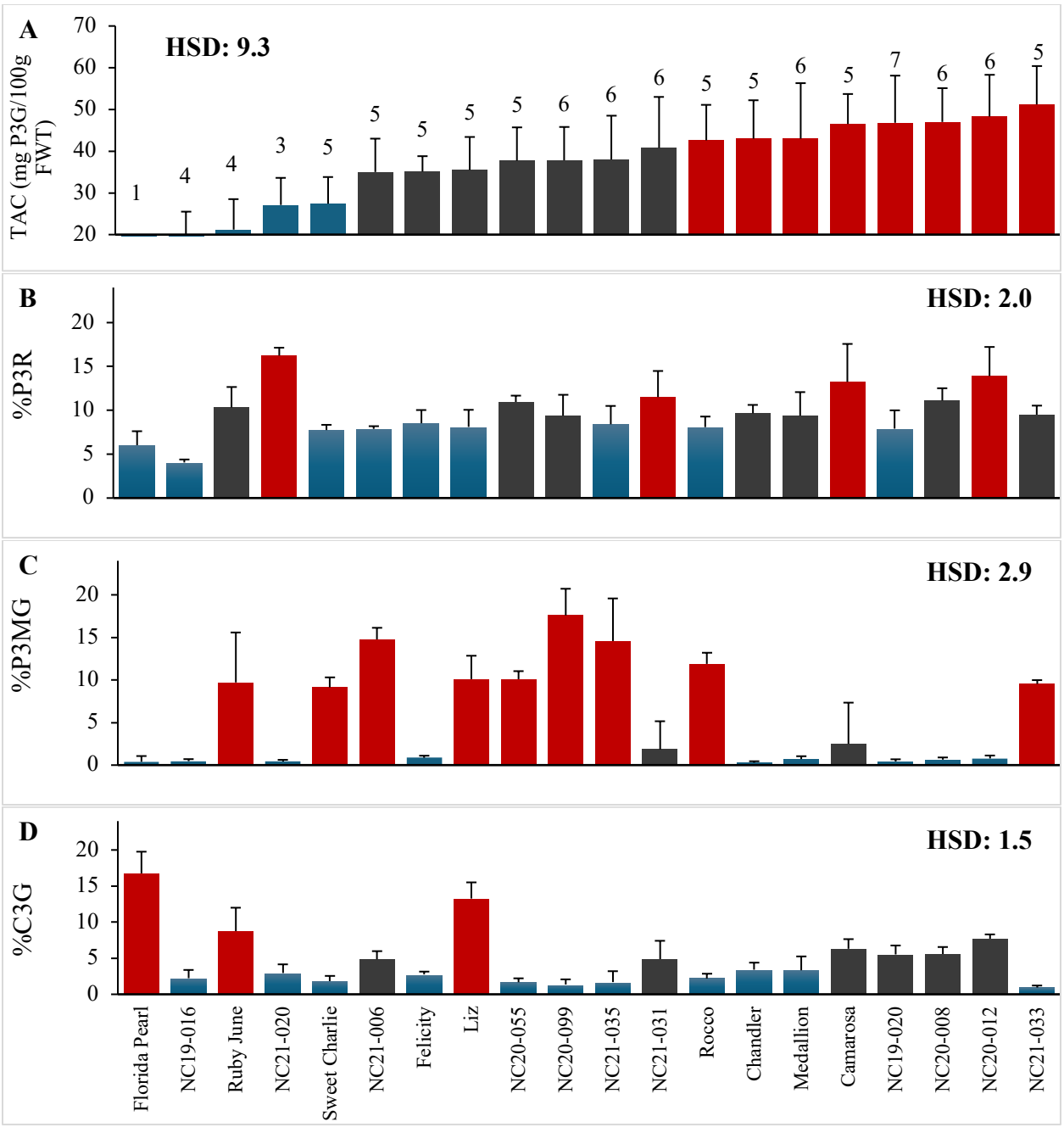

Fig. 2. Total anthocyanin content (TAC), fruit color gradient ratings (A), and relative amounts (% of TAC) of pelargonidin-3-O-rutinoside (P3R) (B), pelargonidin-3-O-(6”-malonylglucoside) (P3MG) (C), and cyanidin-3-O-glucoside (C3G) (D) among North Carolina strawberry genotypes and commercial cultivars. Fruit color was assessed using RosBREED phenotyping protocols, where 1 is white and 9 is very dark red. Bars represent mean values, with standard deviation bars indicating variation across replications (harvest dates). Fruit was collected from April–June 2023, with each genotype harvested on three dates as fruit became available. Significance was determined using Tukey’s HSD at $P < 0.05$, with the HSD threshold displayed in each panel. Differences between means exceeding the HSD values are statistically significant. Bar colors represent relative values within each dataset, with red indicating highest, blue indicating lowest, and black representing intermediate values.

https://doi.org/10.71318/r4s8ab07

References Cited

Aaby K, Mazur S, Nes A, and Skrede G. 2012. Phenolic compounds in strawberry (*Fragaria ×ananassa* Duch.) fruits: Composition in 27 cultivars and changes during ripening. Food Chem. 132(1):86–97. https://doi.org/10.1016/j.foodchem.2011.10.037.

Cayo YP, Sargent S, Nunes C, and Whitaker V. 2016. Composition of commercial strawberry cultivars and advanced selections as affected by season, harvest, and Postharvest Storage. HortScience. 51(9):1134–1143. https://doi.org/10.21273/hortsci10996-16.

Chaves-Silva S, Santos AL, Chalfun-Júnior A, Zhao J, Peres LEP, and Benedito VA. 2018. Understanding the genetic regulation of anthocyanin biosynthesis in plants – tools for breeding purple varieties of fruits and vegetables. Phytochem. 153:11–27. https://doi.org/10.1016/j.phytochem.2018.05.013.

Davik J, Aaby K, Buti M, Alsheikh M, Šurbanovski N, Martens S, Røen D, and Sargent DJ.2020. Major-effect candidate genes identified in cultivated strawberry (*Fragaria ×ananassa* Duch.) for ellagic acid deoxyhexoside and pelargonidin-3-o-malonylglucoside biosynthesis, key polyphenolic compounds. Hortic Res. 7(1), 125. https://doi.org/10.1038/s41438-020-00347-4.

Dzhanfezova T, Barba-Espín G, Müller R, Joernsgaard B, Hegelund JN, Madsen B, Larsen DH, Vega MM, and Toldam-Andersen TB. 2020. Anthocyanin profile, antioxidant activity and total phenolic content of a strawberry (*Fragaria × ananassa* Duch) genetic resource collection. Food Biosci. 36.100620. https://doi.org/10.1016/j.fbio.2020.100620.

Fredericks CH, Fanning KJ, Gidley MJ, Netzel G, Zabaras D, Herrington M, and Netzel M. 2012. High-anthocyanin strawberries through cultivar selection. J. Sci. Food Agric. 93(4):846–852. https://doi.org/10.1002/jsfa.5806.

Haynes B. 2024. Characterization of fruit composition of North Carolina Germplasm. MS Thesis, North Carolina State University, Raleigh, NC, USA. Available online: https://repository.lib.ncsu.edu/items/34371ec3-5636-4196-81d6-c5926e7dd879.

Hoffmann M, McWhirt A, Samtani J, Moore C, Schnabel G, Tregeagle D, Dankbar H, Fernandez G, Simmons C, Perkins-Veazie P, and Poling B. 2024. Southern Regional Strawberry Plasticulture Production Guide, NC State Extension Publications. Available online: https://content.ces.ncsu.edu/southern-regional-strawberry-plasticulture-productionguide.

Lin Y, Jiang L, Chen Q, Li Y, Zhang Y, Luo Y, Zhang Y, Sun B, Wang X, and Tang H. 2018. Comparative Transcriptome Profiling Analysis of Red- and White-Fleshed Strawberry (*Fragaria ×ananassa*) Provides New Insight into the Regulation of the Anthocyanin Pathway. Plant Cell Physiol. 59(9):1844-1859. https://doi.org/10.1093/pcp/pcy098.

Lopes da Silva FL, Escribano-Bailón MT, Pérez Alonso JJ, Rivas-Gonzalo JC, and Santos Buelga C. 2007. Anthocyanin pigments in strawberry. Food Sci. Technol. 40(2):374-382. https://doi.org/10.1016/j.lwt.2005.09.018.

RosBREED. 2024. Strawberry Phenotyping Protocol. Available online: https://www.rosbreed.org/sites/default/files/files/RosBREED_2010-Phenotyping_protocol_Fragaria-slides.pdf.

Perkins-Veazie P, Pattison J, Fernandez G, and Ma G. 2016. Fruit quality and composition of two advanced North Carolina strawberry selections. Int. J. Fruit Sci. 16(1):220-227. https://doi.org/10.1080/15538362.2016.1219289.

Samtani JB, Rom CR, Friedrich H, Fennimore SA, Finn CE, Petran A, Wallace RW, Pritts MP, Fernandez G, Chase CA, Kubota C, and Bergefurd B. 2019. The status and future of the strawberry industry in the United States. HortTechnology. 29(1):11-24. https://doi.org/10.21273/HORTTECH04135-18.

Tulipani S, Marzban G, Herndl A, Laimer M, Mezzetti B, and Battino M. 2011. Influence of environmental and genetic factors on health-related compounds in strawberry. Food Chem. 124(3):906–913. https://doi.org/10.1016/j.foodchem.2010.07.018.

Vinson EL, Perkins-Veazie PA, Blythe EK, Coneva ED, and Price MD. 2022. Five- year Evaluation of Selected Strawberry (*Fragaria ×ananassa* Duch.) Cultivars for Improved Sustainability of the Strawberry Industries in Alabama and the South Atlantic Region of the United States. J. Am. Pom. Soc. 76(3):114-124.

Whitaker VM, Dalid C, Osorio LF, Peres NA, Verma S, Lee S, and Plotto A. 2023. Florida Pearl® 'FL 16.78-109' Pineberry. HortScience. 58(1):143-146. https://doi.org/10.21273/HORTSCI16951-22.

