Comparison of anthocyanin levels in the USDA tart cherry collection

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Introduction

Cellular oxidation is associated with several chronic diseases in humans. Dietary antioxidants inhibit harmful oxidation and promote human health. Anthocyanins are potent antioxidants that occur naturally in fruit and vegetables as red, purple, and blue pigments. They provide many health benefits, including anti-viral, anti-cancer, and anti-inflammatory properties (Wong, 2017). Anthocyanins are known to protect against diabetes, promote healthy sleep patterns with increased levels of melatonin, provide arthritis relief with decreases in swelling; they are even known to slow down the progression of cancer cells. A food source that is known to have an abundance of anthocyanins are cherries, both tart (*Prunus cerasus*) and sweet (*Prunus avium*). Though similar in color and appearance, cherries differ in the anthocyanins present in fruit, with cyanidin 3-glucosylrutinoside as the primary form in tart cherries and cyanidin 3-rutinoside as the primary form in sweet cherries (Kirakosyan et al, 2009). Tart cherry production in the US is valued around $90.8 million (NASS, 2017), with 'Montmorency' as the primary cultivar (Charles, 2013).

1. Our first objective was to measure anthocyanin content in tart cherry genetic resources maintained in Geneva, NY.
2. Our second objective was to compare anthocyanin levels of processed tart cherry juice to different tart cherries.
3. Our last objective was to see if there is a correlation between the type of cherries and fruit quality traits such as acidity, pH, and sweetness.

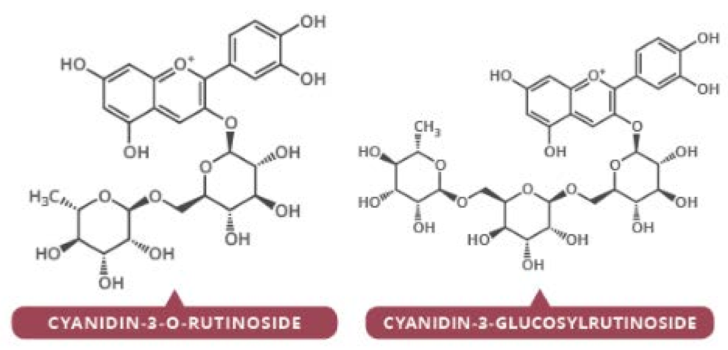

Figure 1. Prominent anthocyanins in sweet (left) and tart (right) cherries

Materials and Methods

Prunus accessions from the USDA-ARS Plant Genetic Resources Unit Tart Cherry Collection in Geneva, NY from the years 2011, 2013, and 2014 cherries were harvested at maturity, frozen and stored at -80°C. Juice samples and sweet cherries from were collected in 2018. Three cherries from each sample in each year were crushed to remove pits, and then finely ground using a GenoGrinder at 1100 strokes/min for 1.75 minutes. 0.5g of ground sample was suspended in a 1.5mL 70% methanol and 2% formic acid solution. Samples were shaken at 1450 strokes/min for 30 minutes, then centrifuged at 15,000 rpm for 10 minutes. Supernatant was filtered with a 0.2 µm nylon filter. Juice samples were filtered with a 0.2 µm filter before injecting. A 5 µm 4.6 x 250 column was used in the separation using a shortened reverse-phase HPLC method from Zhang et al. (2010) with a diode array detector. Chromatographs were analyzed using EZChrom elite software. Cyanidin 3-glucosylrutinoside and cyanidin 3-rutinoside were quantified at 525 nm, with retention times at 15 and 19 minutes, respectively. Data was converted to µg/g for fruit, or µg/ml for juice, following a standard curve for cyanidin 3-galactoside ($R^2 > 0.99$).
For each season, fruit quality traits were collected, including brix, titratable acidity (TA), and fruit and pit weights. Accessions were genotyped using GBS (Elshire et al. 2011) using the TASSEL 5.0 pipeline (Glaubitz et al. 2014).

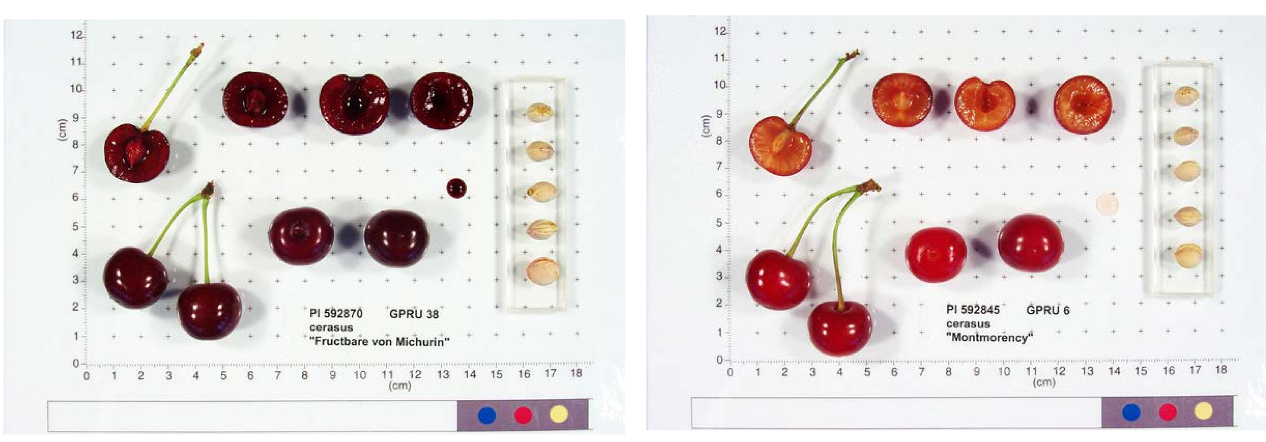

Figure 2. Anthocyanins in ' Fructbar von Michurin' (1333.0 µg/g, left) and 'Montmorency' (99.2 µg/g, right)

Results

In 2011, the overall average levels of cyanidin 3-glucosylrutinoside was 575.8 µg/g, while the average levels of cyanidin 3-rutinoside was 413.3 µg/g. In 2013, both levels of anthocyanins decreased; cyanidin 3-glucosylrutinoside had an average of 338.4 µg/g and 137.9 µg/g for cyanidin 3-rutinoside. In 2014, the mean levels for cyanidin 3-glucosylrutinoside spiked up to 622.6 ug/g while cyanidin 3-rutinoside remained around 285.4 µg/g. The overall cherry cultivars with the most cyanidin 3-glucosylrutinoside levels in the three years combined was 'Stevensbar' with 1466.0 µg/g, followed closely by 'Almaz OP', 'Sumadinka', and 'Fructbare von Michurin'. The overall cherry cultivars with the most cyanidin 3-rutinoside levels in the three years combined was 'Almaz OP' with 1886.9 µg/g, followed by 'Shubinka'. There were weak to moderate seasonal correlations (r = 0.3-0.4) for each anthocyanin. The levels of both anthocyanins in the commercial juice decreased the closer the juice got to their expiration dates. However, regardless of expiration date, the levels of cyanidin 3-glucosylrutinoside were significantly higher than cyanidin 3-rutinoside. PCA for genotypes showed greater genetic variation for *P. cerasus* than *P. avium*. We identified two potentially misclassified *P. cerasus* accessions grouping with *P. avium*.

Results Cont.

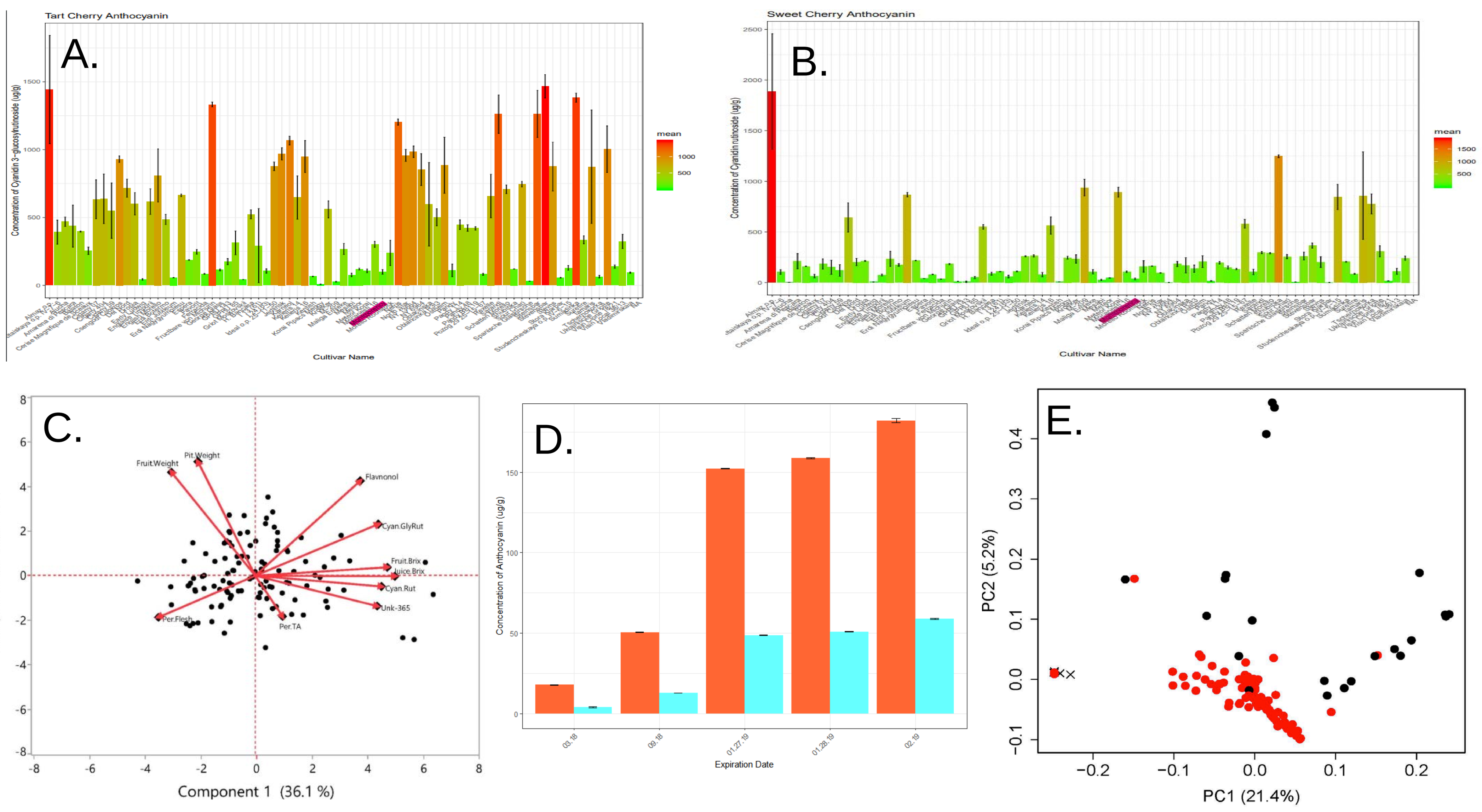

Figure 3. Results from tart cherry study
A. Average levels of glucosylrutinoside in *P. Cerasus* cultivars from 2011, 2013, 2014. 'Montmorency' is highlighted
B. Average levels of rutinoside in *P. cerasus* cultivars from 2011, 2013, 2014. 'Montmorency' is highlighted
C. PCA of fruit quality traits
D. Cyan-rut (blue) and Cyan-glyrut (orange) levels from commercial juice by expiration date
E. PCA showing genetic diversity in *P. cerasus* (red), *P. avium* (Xs), and wild *Prunus* (black

Discussion

The anthocyanin levels varied greatly in each season. Cultivars that could produce relatively consistent levels of anthocyanins despite weather variations season to season would be beneficial for commercial production. Although 'Montmorency' is more broadly utilized in the US, it did not have consistent levels of anthocyanins, with lower concentrations relative to other accessions. Despite these low concentrations, people have been able to experience the health benefits associated with the tart cherry consumption. It is possible that cultivars with higher levels of anthocyanins could also increase the beneficial properties of tart cherry products. It is important to note that there are many other characteristics that must be considered when producing commercial cherries. These properties include, but are not limited to: firmness, sweetness, acidity levels and most importantly hardiness, or ability to grow in different weather conditions. Since the primary anthocyanin in *P. cerasus* is cyanidin 3 -glucosylrutinoside, it was predicted that all of the cultivars labeled cerasus would have higher concentrations of cyanidin 3 -glucosylrutinoside than of cyanidin 3 -rutinoside. However, there were several cultivars that fit the chemical profile of *P. avium,* with significantly higher concentrations of cyanidin 3-rutinoside. It is possible that those *P. cerasus* cultivars could potentially be labeled incorrectly. Genotypes for two *P. cerasus* accessions confirmed a misclassification. In addition, there was one cultivar, 'Almaz OP' that had high concentrations of cyanidin 3 -glucosylrutinoside one year, but showed high concentrations of cyanidin 3-rutinoside the following year. The most recently produced cherry juice had higher cyanidin 3-glucosylrutinoside concentrations than that of 'Montmorency' from our collection, and could be a result of fruit origin or processing. As anthocyanin content decreased in juice significantly overtime, utilizing cultivars with higher initial levels of anthocyanins could extend the nutritional value of commercial juices.

Figure 4. 'Montmorency' by Thomas Chao

Conclusions

- *Additional tart cultivars beyond 'Montmorency' should be further evaluated for fruit and nutritional qualities to increase value of tart cherry products*
- *Follow up research should be done with 'Almaz O.P'*
- *A larger sample size of sweet cherries and other Prunus species would allow for more extensive comparative analysis*

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