## Progress Report for 2024 SRSFC Research Grant R-23

# Chlorogenic Acid Content in Rabbiteye Blueberry (*Vaccinium virgatum*) Germplasm after Harvest and Storage

# **Public Abstract:**

Chlorogenic acid (CGA), found in high amounts in apple, strawberry, artichoke and blueberry, is a phenolic acid with phytochemical benefits. In humans, CGA appears to regulate glucolipid metabolism, providing antioxidant and anti-inflammatory benefits. Ripe fruit from 16 blueberry cultivars and genotypes with rabbiteye (*V. virgatum*) species background were harvested from one to two locations and held at 0, 14, and 21 days storage at 2 °C (34 °F). Blueberries from the day 0 harvest were analyzed for CGA (others are underway in analysis). Cultivars and genotypes varied widely in CGA, from 100 to 300 mg/100 g. Values are within range of other reports, although lower in CGA than cultivars harvested from North Carolina.

Principal Investigator	Co-Investigator #1	<b>Co-Investigator</b>	Co-Investigator #3
		#2	
Penelope Perkins-Veazie	Sushan Ru	Marlee Trandel-Hayse	Massimo Iorizzo
N.C. State University	Auburn Univ.	Auburn Univ.	N.C. State University
600 Laureate Way	120 Funchess Hall	121 Funchess Hall	600 Laureate Way
Kannapolis, NC 28081	Auburn, AL 36849	Auburn, AL 36849	Kannapolis, NC 28081
penelope_perkins@ncsu.edu	sushan.ru@auburn.edu	mat0141@auburn.edu	miorizz@ncsu.edu

## **Objectives:**

The primary objective of this project is to determine the amount of chlorogenic acid in germplasm of *Vaccinium virgatum* (rabbiteye blueberry). The subobjective of this work is to follow the retention of chlorogenic acid during low temperature storage of fresh blueberries. Justification and Description:

In previous work, rabbiteye blueberries have been reported to be unusually high in chlorogenic acid (CGA) (Yousef et al., 2013; 2016). This phenolic acid is associated with chlorogenic acid intake and application, including anti-inflammatory, anti-diabetic, and anti-cancer properties (Kim and Park 2019). Rabbiteye blueberries may have a thicker peel than southern or northern highbush types; flavonoids are mostly in the peel rather than the pulp of blueberry (Ribera et al., 2010), which could indicate that more phenolic acids are also present in rabbiteye blueberries.

Blueberries from a North Carolina field of varied genotypes and cultivars were screened for CGA in 2022 (Figure 1). Of the 51 cultivars sampled, 7 of the 8 rabbiteye genotypes (Titan, Premier, Ira, Vernon, Columbus, Alapaha, Brightwell) were high in CGA. Rabbiteye blueberries are robust plants, with more resilience to high temperature, drought, and heavy soils than southern highbush genotypes (Krewer and NeSmith 2006). While chlorogenic acid content

offers an extra health attribute in blueberry consumption (McDougall et al. 2020), changes in CGA of blueberries after cold storage are unknown. Increased or decreased CGA could affect postharvest life and/or flavor. The purpose of this project is to follow chlorogenic acid in rabbiteye cultivars and advanced selections after harvest and fresh market storage.

#### **Materials and Methods**

Of the 50-60 selections of rabbiteye material at Auburn desired for screening of CGA, Eleven established cultivars, including 'Tifblue', 'Vernon', 'Krewer', 'Ochlocknee', 'Alapaha', and 6 advanced selections were evaluated for day 0 material. Fully blue fruit (15-60 berries per selection), free of injury or defects, were used. Additional fruit of the cultivars and selections that were held for 2 and 3 weeks storage at 2-3 °C at Auburn University are still being analyzed for CGA. Blueberry fruit samples were frozen and held at -20 °C at Auburn University, then shipped to PHHI on dry ice.

All samples were divided into 3 replicates per cultivar/selection and freeze dried for 10-14 days using a VirTis LyoTroll (SP Scientific, Warminster, PA, USA. Freeze dried berries were ground with a Geno grinder (SPEX, New Jersey) for 2 min at 1200 strokes/sec to obtain a uniform small particle size. Independent replicates of about 20 mg powder were mixed with 1.5 ml acidified methanol (formic acid:methanol:deionized water, 1:60:39, v/v/ v) in 2 ml microfuge tubes, vortexed for 30 sec (Benchmark 1000, Grainger), sonicated for 15 min (Branson Ultrasonicator) and centrifuged for 20 min at 13,000 x g (microcentrifuge Model 5425R, Eppendorf). One extraction was found to capture 98% of the phenolic acids. Supernatants were combined, and 1 ml aliquots filtered through a 0.20  $\mu$ M PTFE filter into

amber HPLC vials. A nitrogen flush will be done of the headspace and vials capped with screw top lids.

High performance liquid chromatography (HPLC) was used to determine CGA following the method of Kim et al. (2015). A high performance liquid chromatography system (Elite LaChrom, Hitachi Ltd., Tokyo, Japan) equipped with autosampler, diode array detector, and binary solvent delivery manager was used to analyze phenolic compounds. Samples (10 µl) were injected onto a reversed phase C18 column (Synergi Hydro-RP 80Å 4.6 x 6 mm, Phenomenex, Torrance, CA, USA) held at 30 °C. Mobile phases were 5% formic acid (solvent A) and 100% methanol (solvent B), with a flow rate of 16.7 mL/min. The gradient system was 0–5 min, 90% A; 5–15 min, 85% A; 15–20min, 80% A; 20–25 min, 75% A; 25–45 min, 70% A; 45–47 min, 40% A; 47–60 min, 90% A. Hydroxycinnamic acids will be detected at 320nm. Standard curves of gallic acid, ferulic acid, and chlorogenic acid were used to identify and quantify phenolic acids.

Statistical analysis will be done using a univariate analysis of variance (ANOVA) with SAS v. 9.4., with 3 replicates per sample. Means will be separated using Tukey's honestly significant difference test (HSD) at the 95% significance level.

## **Results and Discussion**

We were able to complete analysis only on day 0 fruit in 2024, due to unexpected delays in collecting and assaying the blueberries. Currently, only chlorogenic acid content has been quantified. HPLC analysis of blueberries stored at other timepoints will be finished by February of 2025.

Considerable variation in CGA was found among cultivars and genotypes with *V. virgatum* background grown in Alabama (Fig 1). 'Powderblue' was highest and selection T-2467 was lowest in CGA (358 and

106 mg/100 g dwt, respectively). Values for 'Premier', 'Vernon', and 'Alapaha' were similar to 2024 CGA cultivar values for blueberries harvested in North Carolina. However, 'Titan' berries had half of the CGA content found in North Carolina berries (600 mg/100 g dwt). 'Pink Lemonade' CGA values were similar to that reported by Grace et al (2019), at 331 mg/100 g dwt. 'Pink Lemonade' is red colored cultivar with a relatively low anthocyanin content (Grace et al., 2019). The CGA values for 'Pink Lemonade' were similar to five blue colored cultivars in that study, indicating that selecting for CGA using berry color may not be useful. Chlorogenic acid content may be regulated by a single gene, as found in a diploid interspecific blueberry population (Herniter et al. 2023). The possibility of CGA loci in *V. virgatum* populations is currently unknown.



Figure 1. CGA mean content of rabbiteye cultivars/genotypes from Alabama. Means are separated by LSD, p<0.10. Different colored bars indicate groupings of means.

# **Budget:**

Labor for extraction, sample preparation, data summary (160 h @\$20/h) \$3000 Supplies (disposables, standards, solvents, HPLC column) \$1800 Shipment of samples \$ 200 Total \$5,000

## Literature Cited

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