

## Southern Region Small Fruit Consortium

### Final Report Research

**Title:** Chlorogenic Acid Content of 50 Blueberry Cultivars

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### Public Abstract:

Blueberry fruit have a number of recognized bioactive compounds that are helpful for human health. Chlorogenic acid (CGA), a phenolic acid often associated with coffee, is unusually high in blueberry fruit. Studies with a few blueberry cultivars indicate that genotype/species may greatly influence relative chlorogenic acid content. In this study, freeze dried fruit from 50 blueberry cultivars, including northern and southern highbush and rabbiteye types, were assayed for CGA. Powders were solubilized in acidified methanol and CGA detected and quantified using HPLC. Large differences were found among germplasm, with values of CGA content, ranging from 133 to 718 mg/100 g dry weight. The high to medium CGA values indicate that blueberries of the three major fresh market genotypes all offer CGA for consumers.

### Introduction

Blueberries are a rich but often overlooked source of chlorogenic acid. Chlorogenic acid (CGA), a cinnamic acid derivative that contributes most of the non-anthocyanin phenolics in blueberry (Kader et al., 1996; Yousef et al. 2013), is most often associated with coffee. A number of positive health aspects are associated with chlorogenic acid intake and application, including anti-inflammatory, anti-diabetic, and anti-cancer properties (Kim and Park 2019).

CGA can have negative effects on blueberry quality, as it reacts with polyphenol oxidase and causes internal browning of blueberries, especially after bruising or juicing (Kader et al. 1997; Moggia et al. 2017; Siddiq and Dolan, 2017).

Yousef et al. (2016) reported variation in chlorogenic acid content (36 to 162 mg/100 g) of seven southern highbush and eight rabbiteye cultivars. Knowing the relative amounts of CGA in

blueberry cultivars can be important for health marketing, for potential issues with bruising from mechanical harvesting, or for processing applications.

### **Objectives:**

The primary objective of this project was to determine the amount of chlorogenic acid in a collection of blueberry germplasm including northern and southern highbush and rabbiteye genotypes.

### **Methods**

Blueberries of 51 cultivars, consisting of 9 rabbiteye, 7 northern highbush, and 34 southern highbush from diverse breeding programs. Plants were grown at the Castle Hayne Research Station, North Carolina Department of Agriculture and Consumer Services (NCDA&CS), NC and were harvested from May to July of 2022. All fruit were precooled to 12-15 °C immediately after harvest and during transit to the Plants for Human Health Institute in Kannapolis and firm, fully blue fruit were frozen at -20 °C. Samples of 30 to 60 berries per cultivar, divided into 3 replicates of 10 to 15 berries per replicate (depending on fruit size) were then freeze dried using a VirTis LyoTroll (SP Scientific, Warminster, PA, USA) and held at -80 °C. These samples will be brought to room temperature then ground with a Geno grinder for 2 min at 1200 strokes/sec to obtain a uniform small particle size.

Three replicates per cultivar, consisting of about 20 mg freeze dried powder, were combined with 1.5 ml acidified methanol (formic acid:methanol:deionized water, 1:60:39, v/v/ v) in 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). The supernatant was transferred to a 5 ml tube and the pellet re- extracted with 1.5 ml solvent as described above, which captures 98% of phenolic acids (Kim et al., 2015). Supernatants were combined, and 1 ml aliquots filtered through a 0.20 µM PTFE filter into amber HPLC vials. A nitrogen flush was done of the headspace and vials capped with screw top lids. Samples were held at -80 °C until analysis.

Sample injections of 20 µL were injected on to a high performance liquid chromatography system (Elite LaChrom, Hitachi Ltd., Tokyo, Japan) equipped with autosampler, diode array detector, and binary solvent delivery manager. Separation of phenolic compounds was done using reversed phase C18 column (Synergi 4m Hydro-RP 80Å, Phenomenex, Torrance, CA, USA) at a column temperature of 30 °C. Mobile solvent phases were 5% formic acid (solvent A) and 100% methanol (solvent B), at a flow rate of 16.7mL/min and a gradient system of 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; 47–60min, 90% A. A chlorogenic acid standard (Sigma, C3878, 5-*O*-caffeoylquinic acid) was used to identify and quantify chlorogenic acid at 320 nm absorbance.

Statistical analysis was done using a univariate analysis of variance (ANOVA) with SAS v. 9.4. Means were separated using Tukey's honestly significant difference test (HSD) at the 95% significance level.

## Results and Discussion

Blueberry fruit had a wide range of chlorogenic acid (CGA) values, from 133 to 718 mg/100 g dwt (Table 2). Standard deviations among cultivars were relatively high, and the resulting Tukey's (HSD) value was also high. The small subsets of northern highbush and rabbiteye had higher mean CGA than southern highbush (Table 1), as was found by Yousef et al. (2016). *V. angustifolium* and *V. darrowii* blueberries have been reported to be unusually high in CGA (Herniter et al., 2023; Rodriguez-Mateos et al., 2012).

Titan was also very high in CGA (Table 2). This blueberry is very large, averaging 4 g compared to the 2.5 g of most of the cultivars. In contrast, Mini Blues, a small fruited cultivar (about 1.5 g) was also quite high in CGA. CGA appears to be a free phenolic acid rather than bound (Pico et al., 2022) and may be associated mostly with blueberry peel (Kader et al., 1996). Relative values among the genotypes and cultivars may be normalized if only peel is used for CGA determinations.

The range of values within the southern highbush group indicates that blueberry cultivar may have as much or more variation than its respective genotype. For instance, the SHB cultivars Biloxi, Santa Fe, and Misty were very high in CGA compared to Jackie and Meadowlark (Table 2). Herniter et al. (2023) and Mengist et al. (2022) have reported QTL for CGA in blueberry fruit and the possible loci involved in its biosynthesis.

Yousef et al. (2016) found that relative values of CGA within a cultivar could differ from 0 to 40% in blueberry fruit with year of harvest. This indicates that environment may have a strong influence on CGA, as would be expected for phenolic compounds. Several years of CGA assay may be needed if blueberry germplasm is being considered for release based on high CGA values.

Table 1. Chlorogenic acid content among blueberry genotypes

Type	Mean
Rabbiteye	401.61a
Northern highbush	335.72b
Southern highbush	270.33c
	HSD 35.34

Means statistically separated by Tukey's HSD test.

Table 2. Chlorogenic acid content among blueberry cultivars.

Cultivars highlighted in blue are rabbiteye and those highlighted in green are northern highbush. All others are southern highbush.

	Mean	Stdev
	<i>mg/100g dwt</i>	
Biloxi	718.65	265.98
Titan	659.52	69.17
Santa Fe	542.60	99.53
Ira	487.10	108.23
Misty	487.11	49.76
Bladen	471.34	104.64
Mini Blues	453.16	12.64
Clockwork	447.25	41.75
Sampson	431.64	53.43
Sweetheart	408.00	55.69
Vernon	402.34	44.08
Columbus	395.03	25.07
BlueRidge	378.39	73.72
Premier	376.78	35.62
Alapaha	357.73	17.44
Brightwell	355.69	15.49
Draper	354.09	20.40
New Hanover	342.08	23.82
Reveille	341.42	43.65
Pender	339.23	68.71
Emerald	338.99	65.26
Pinnacle	323.58	42.01
Croatan	304.17	29.20
Rebel	285.81	29.44
Southern Splendor	271.85	23.93
Star	259.29	52.38
Abundance	257.4	29.7
O'Neal	255.62	19.07
Prince	253.38	22.34
Windsor	241.33	39.83
Farthing	226.32	30.26
Suziblue	225.76	33.76
Jewel	220.32	4.05
Carteret	220.19	78.74
Bluecrisp	219.72	61.08
TopShelf	219.41	15.47
Miss Alice Mae	215.63	41.51

Pearl	211.79	14.89
Indigocrisp	210.98	38.28
Jubilee	205.26	31.74
Camellia	202.11	33.16
Lilly	193.55	51.78
Blue Ribbon	191.15	16.07
Vireo	186.09	12.84
Snowchasher	177.76	28.90
Millenia	176.75	66.38
Ventura	170.70	47.10
Primadonna	167.10	16.10
San Joaquin	147.90	11.20
Jackie	136.88	19.00
Meadowlark	132.89	20.77
HSD	201.03	

### Impact Statement

Blueberry fruit from cultivars representing three genotypes (northern, southern, and rabbiteye) were analyzed for chlorogenic acid content (CGA), a phytochemical useful in human health. Some cultivars of blueberry fruit showed a CGA content that was comparable to dry tealeaves. Values were highly different among the cultivars, with those in the rabbiteye (*V. virgatum*) species generally exceeding cultivars from northern or southern highbush germplasm. Compared to other reports for blueberry fruit, environmental conditions may have a significant influence on total amounts of CGA. This indicates that multiple years of analysis should be done for blueberry cultivars that appear to have high values of CGA. Given the strong genetic component controlling CGA accumulation in blueberry, this result provides guidance to breeders to which lines may harbor gene for high CGA and use them in the breeding program.

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