Proposal Category: **_X__Research** **___Outreach**

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Title:

Metabolite analysis to identify markers for higher fruit firmness and longer shelf-life in southern highbush and rabbiteye blueberry

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Objective:

Identify key metabolites such as specific sugars and acids which can be used as biomarkers for postharvest fruit quality in southern highbush and rabbiteye blueberry.

Justification and Description:

Blueberries are considered "super fruits" having high concentrations of phenolic compounds with nutraceutical capacity (Huang et al., 2012). There are numerous health benefits associated with consuming blueberry fruit, some of which include decreased cardiovascular risk, improved cognitive performance, and decrease in ageing-related damage (Neto, 2007; Basu et al., 2010).

After harvest, blueberries have a shelf-life of 1 to 8 weeks. This duration typically depends on the genotype, method of harvest, and the storage regime (Almenar et al., 2008; Sun et al., 2014; Abugoch et al., 2015). The main causes of decreased fruit quality during postharvest storage is water loss, increase in fruit softening, and decay caused by postharvest pathogens (Li et al., 2011; Mehra et al., 2013; Paniagua et al., 2013). Although breeding efforts to improve fruit quality are concentrated towards increasing fruit firmness and total soluble solids, currently there is no information on the important metabolites (individual sugars and acids) that may predict fruit quality. Further there is no information of how fruit quality changes over time during postharvest storage, which is important because of the extended time window before the fruit reach the consumer. If we are able to identify sugars and acids that are key determinants of longer shelf-life, this information can be used in breeding efforts to select for cultivars with improved fruit quality attributes. This is especially important because wholesale buyers and consumers pay attention to the appearance and firmness of fruits, which are major factors associated with fruit quality (NCSU Extension [Boyette et al.]; Maclean and Nesmith, 2011). This approach to identify key metabolites influencing fruit quality and improved agronomic traits has been applied to tomato (Gómez-Romero et al., 2010), peach (Lombardo et al., 2011), grapes (Degu et al., 2014), and strawberry (Zhang et al., 2011).

In Georgia and in the southeastern US, two main types of blueberries are commercially grown: southern highbush (species complex between *Vaccinium corymbosum* L. and *V. darrowii* Camp.) and rabbiteye (*V. virgatum* Aiton) blueberry. Southern highbush and rabbiteye blueberry fruit have been shown to vary in postharvest quality, with rabbiteye blueberry genotypes displaying higher values of skin puncture, berry firmness, carbohydrates, and fiber content (Silva et al., 2005). Saftner et al. (2008) examined instrumental fresh fruit quality measurements of ten highbush and two rabbiteye cultivars grown in New Jersey and reported higher variations associated with cultivar differences than with species differences.

Preliminary Results*:*
Some of our own preliminary data show that fruit quality attributes differ as much among cultivars as between southern highbush and rabbiteye blueberry species. However, we have not been able to identify significant correlations between fruit quality parameters and shelf-life. Fruit are an extensive reservoir of metabolites and measuring the primary chemistry such as total soluble solids (TSS) and titratable acidity (TA) may not be reflective of changes occurring in fruit quality after harvest and during postharvest storage.

Therefore, to determine specific sugars and acids in cultivars that vary in firmness and shelf-life we plan to measure the major sugars: sucrose, fructose, glucose; and the major organic acids: malic acid, citric acid and quinic acid. Our preliminary data from fruit collected in 2015 indicate that quinic acid content is higher in fruit with higher firmness (compression): Suziblue and Titan, compared to fruit with lower firmness: Rebel and Premier. Whether quinic acid content correlates with better fruit quality during storage requires further investigation with multiple varieties that differ in storage attributes. Further, these data provide evidence supporting the hypothesis that specific metabolites may serve as markers correlated with postharvest fruit quality. Thus, our proposal plans to test to what degree specific metabolites, such as quinic acid, can be used to predict storage attributes among cultivars.

Significance
Blueberry is an important crop in Georgia and throughout the southeastern US. Phenotypic variation within and among species for fruit quality characteristics is present as shown by previous research and our preliminary results. Increased knowledge of changes in sugars and acids in varieties that differ in fruit quality would help in identifying metabolites that can serve as markers that predict blueberry quality. This information can be incorporated into southeastern blueberry breeding programs and also help with selection of new cultivars and parents. Overall, knowledge from this project would benefit blueberry growers, consumers, and the industry by providing material with increased quality.

Description of Procedures:

Fruit material
The purpose of this study was to determine sugar and organic acid profiles of several southern highbush and rabbiteye cultivars from fruit collected in 2017. For southern highbush blueberry, Suziblue, Rebel, Emerald, Farthing, and Miss Lilly will be used. For rabbiteye, cultivars Titan, Brightwell, Alapaha, Premier, and Powderblue will be evaluated. Samples were collected at the green, pink, ripe, PH5, PH12, and PH21 stages. This project is in progress; however, due to the COVID-19 and the lab being shut down, we only evaluated the two

replications of Suziblue and Rebel so far. In this section, we discuss the result from the only two replications of Suziblue and Rebel. The remaining cultivars are currently being evaluated.

Sugar/Acid profiling

Identification of compounds was performed using Gas chromatography-mass spectrometry (GCMS) equipped with 5973 quadrupole mass spectrometer detector (Agilent Technologies 6890N Network GC system). An HP-5 fused capillary column (J&W Scientific, Fulsom, CA, USA) was employed. The methods set up is similar as in the GC-flame ionization detector (GC-FID) described below.

The quantification of compounds was performed by using the GC-FID (GC-2014; Shimadzu, Japan). The extraction protocol was done according to Chapman and Horvat (Chapman Jr and Horvat 1989) with some modification. Around 100-150 mg of frozen grounded samples were extracted with 100% methanol, followed by centrifugation at 22000 g for 30 minutes. After that, 100 µl of supernatant was transferred into the GC-vial. Supernatants were evaporated under the nitrogen gas at 45 °C. After that, 50 µl of methoxyamine-HCl (20 mg metoxyamine in 1 ml pyridine) was added to each sample. Then samples were heated at 50 °C for 30 minutes for making the oxime derivatives. Finally, derivitization of compounds were done by adding the 100 µl of N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) + 1% TMCS (trimethylchlorosilane) and heated at 50 °C for 30 minutes.

In the GC-FID, helium was used as a carrier gas. The initial temperature of the oven was set up at 150 °C for 1 minute. Then the oven temperature was ramped at 4 °C to 190 °C, 1 min at 190 °C. After that, the temperature was ramped at 0.5 °C per minute to 210 °C. The temperature was held for 1 minute at 210 °C. Finally, the temperature was increased to 260 °C at 10 °C per minute and hold for 15 minutes at 260 °C. Split ratio of 20:1 was used. So far, we have quantified the concentration of malic acid, citric acid, quinic acid, glucose, fructose, and sucrose. We also prepared the standard curve for each of these compounds (Supplemental Fig. 1). The identification and quantification of other compounds like shikimic acid, glutamine, Myo-inositol, oxoproline, chlorogenic acid, succinic acid, and xylose are in progress.

Results

Organic acid: Concentration of malic acid was higher in the green fruit stage, then decreased during ripening and PH5 stages and increased in PH12 and PH21 in Suziblue (Figure 1). In 'Rebel' the concentration of malic acid was similar during ripening and postharvest stages. Comparison between 'Rebel' and 'Suziblue' indicated higher malic acid only at PH21 than Rebel. Concentration of the two other acids, citric and quinic was during early ripening stages and declined in ripe fruit and at postharvest stages. Further, Suziblue had more citric acid at the green, pink, and PH21 compared with Rebel (Figure 1), however, quinic acid was not significantly different between Rebel and Suziblue. Overall, Suziblue increased malic acid by 0.28 fold at PH21, and citric acid by 0.8, 1.16, and 1.6 fold at the green, pink, and PH21, respectively, compared to Rebel (Figure 1).

Sugars: The concentration of sugars which included fructose, glucose and sucrose displayed opposite trends than acids with lower concentrations during initiation of ripening and increasing concentrations in ripe, PH5, PH12, and PH21 stages (Figure 2). The only expection was sucrose concentration which was lower at PH21 than ripe stage. The concentration of fructose, glucose, and sucrose at green and PH21 was more significant in the Rebel than to Suziblue (Figure 2). Compared with Suziblue, Rebel had higher fructose concentration by 0.62- and 0.41-fold,

glucose by 0.65- and 0.37- fold, and sucrose by 0.59- and 0.50- fold at the green and PH21 stages, respectively (Figure 2).

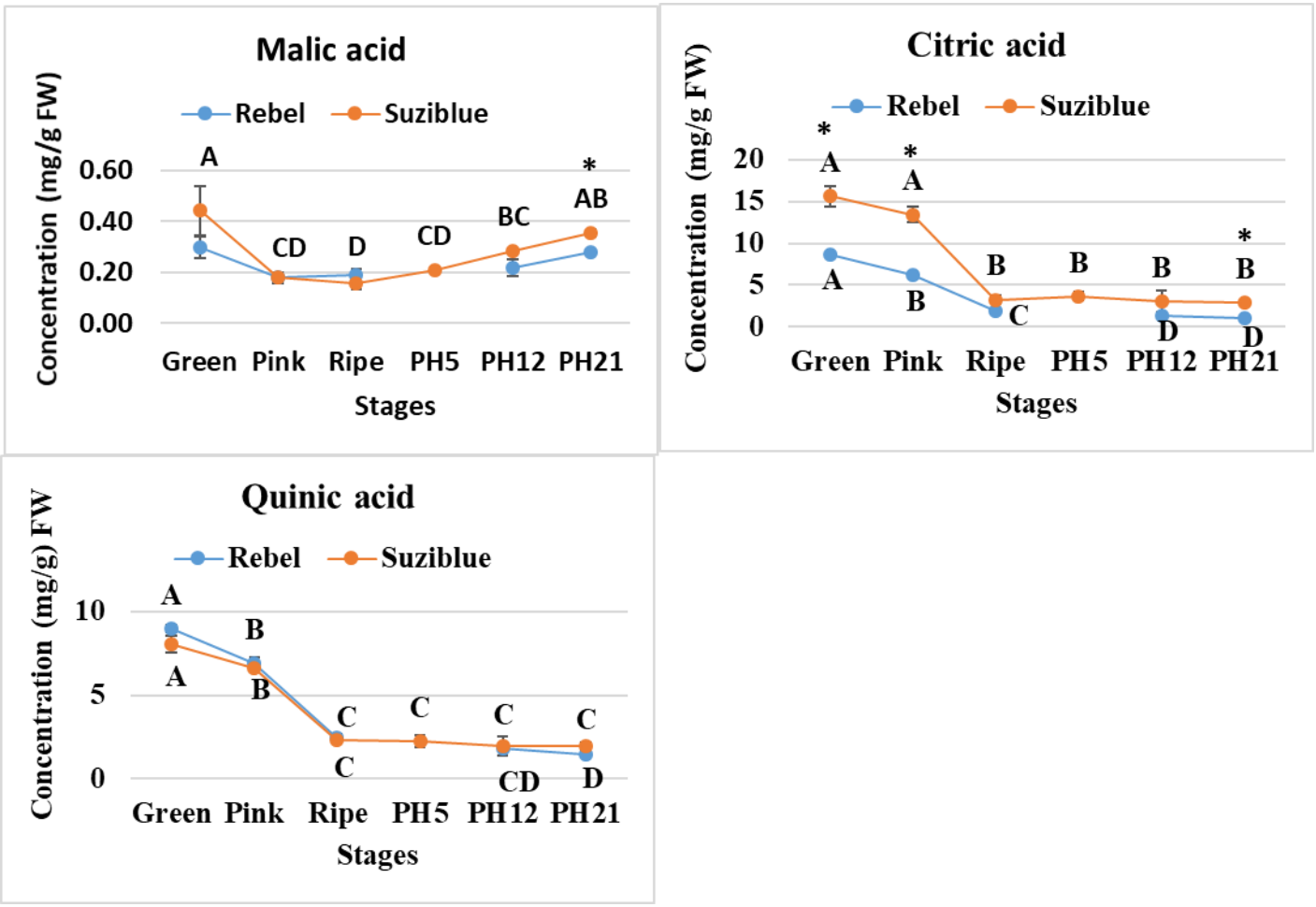


Figure 1: Concentration of malic acid, citric acid, and quinic acid in Rebel and Suziblue at different ripening and postharvest stages. Different uppercase letters at different ripening and postharvest stages are significantly different by Fischer's LSD at a 0.05% level. The asterisk symbols indicate a significant difference between Suziblue and Rebel, respectively ($\alpha = 0.05$)

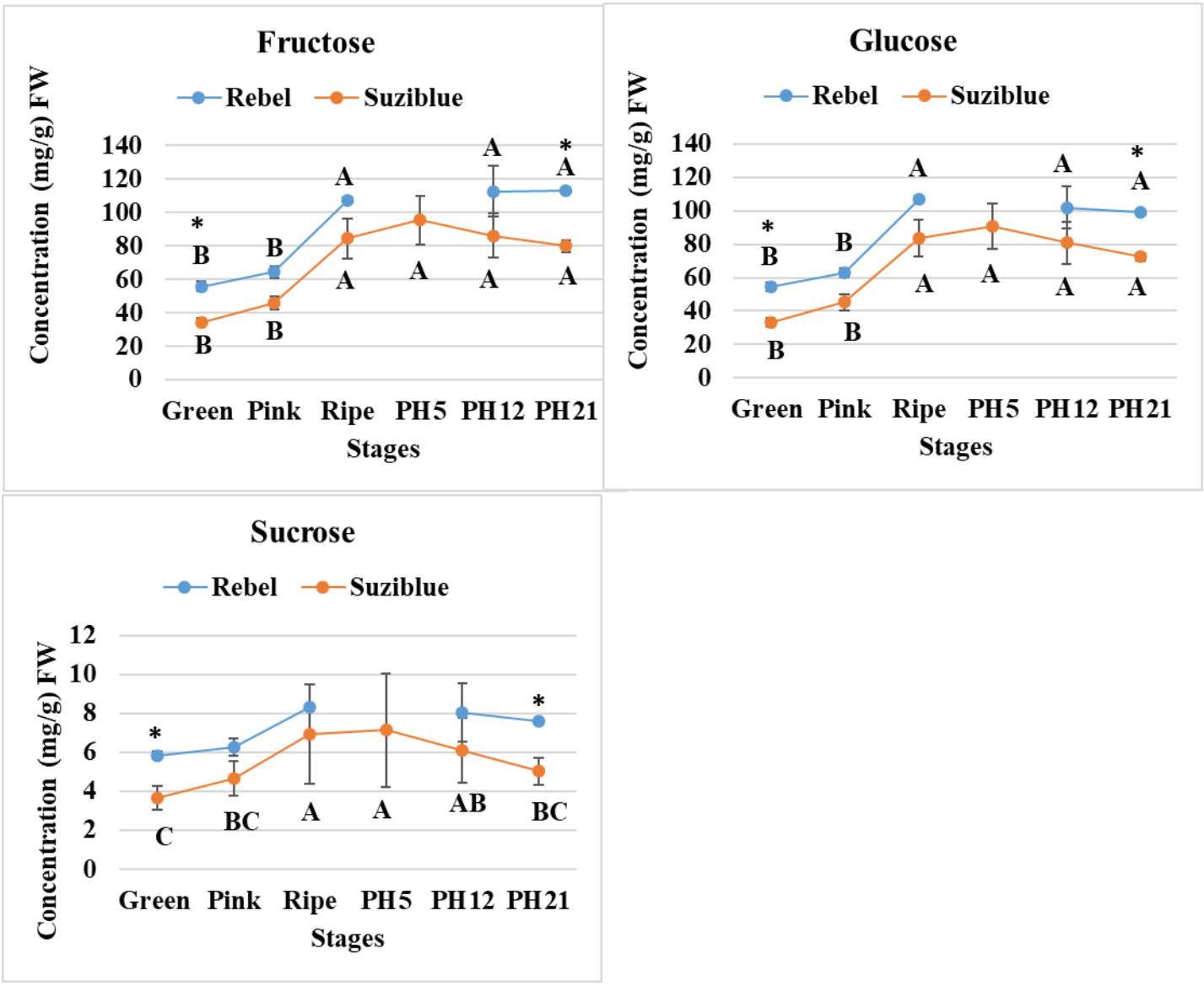
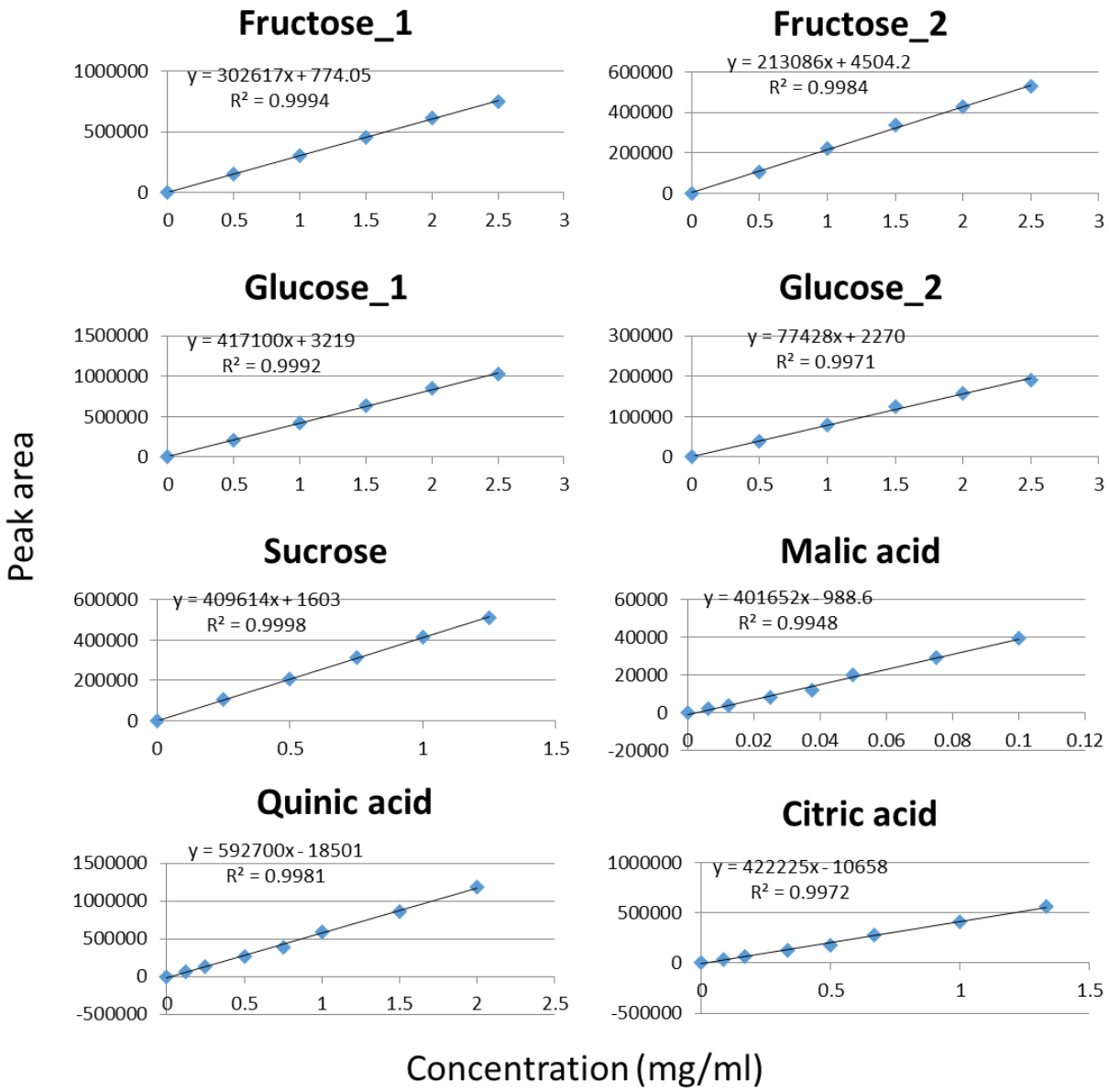


Figure 2: Concentration of fructose, glucose, and sucrose in Rebel and Suziblue at different ripening and postharvest stages. Different uppercase letters at different ripening and postharvest stages are significantly different by Fischer's LSD at a 0.05% level. The asterisk symbols indicate a significant difference between Suziblue and Rebel, respectively ($\alpha = 0.05$)



Supplementary figure 1: Standard curve of fructose, glucose, sucrose, malic acid, quinic acid, and citric acid. In chromatogram two peak detected for fructose and glucose. Each standard curve corresponds with that peak.

Conclusion:

Our data did not reveal differences in quinic acid as expected between cultivars that vary in firmness from samples collected in 2017 from Suziblue and Rebel. Rebel had lower firmness compared with Suziblue in both years 2015 and 2017. Rebel had decreased shelf-life in 2015 compared with Suziblue, however in 2017, both these cultivars had similar shelf life for about a month after harvest. Samples were collected from two separate farms in 2015 (UGA Research farm in Alapaha) and 2017 (commerical farms), and thus metabolic variations could be a reflection of underlying environmental changes. We plan to still evaluate other cultivars and it is

still possible that variation in firmness of shelf life could be cause by more that one metabolic variation.

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