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Title:
Metabolic evaluation of sugars and acids as predictor of fruit quality in southern highbush and rabbiteye blueberry during postharvest storage

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Objective:

The objective of the proposal was to evaluate postharvest fruit quality and shelf-life attributes of established and newer southern highbush and rabbiteye varieties. Further, we planned to determine if the content of specific sugars and acids to see if they can be a useful predictor of fruit quality. The results obtained from this proposed study can increase knowledge and help to breed blueberry cultivars with better fruit quality during extended storage. Additionally, comparisons across emerging varieties can help growers and packers better match varieties and their intended markets.

Justification and Description:

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) blueberries. In 2016, US produced 269,257 tons of blueberries in 92,800 acres (USDA-NASS, 2017) with Georgia among the leading producers in the country. With high production it becomes important to manage postharvest practices to extend the quality of fruit for a longer duration. Wholesale buyers and consumers pay attention to the appearance and firmness of fruits, factors associated with fruit quality (NCSU Extension [Boyette et al.]; Maclean and Nesmith, 2011). Even though blueberry is emerging as such an important commodity currently, very little information is available about factors that influence fruit quality attributes during storage.

Southern highbush and rabbiteye fruit have been shown to have variations 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 variations associated with cultivar differences, rather than species differences. Most recently, work at the University of Georgia (Itle and NeSmith, 2016) with detailed analyses of several southern highbush and rabbiteye varieties has shown considerable variation among varieties in several fresh fruit quality traits. These analyses were made on a one-time basis for the fruit upon harvest, but data for postharvest quality over time were not obtained, so no assessment of shelf-life was made. Therefore, more information on postharvest quality over time is needed for blueberry genotypes cultivated in the Southeastern U.S.

Some of our own preliminary data show that fruit quality attributes differ between cultivars rather than between southern highbush and rabbiteye. Further we have evaluated their quality during postharvest storage and our findings indicate that differences exist among cultivars in their storage capabilities. Cultivars such as Suziblue and Titan have higher compression values and perform better during postharvest storage than cultivars such as Rebel, Premier and Powderblue (Fig. 1). On an average, fruit that were sampled from various supermarket stores were softer in texture in comparison to some of the new southern highbush and rabbiteye cultivars even after 3 weeks of postharvest storage (Fig. 1). These data indicate a potential for improvement in postharvest handling and maintenance of fruit quality after harvest. Data from such studies is vital for growers, packers, and marketers to better understand how they should handle varieties in time and space to offer consumers the best fruit quality and shelf-life possible.

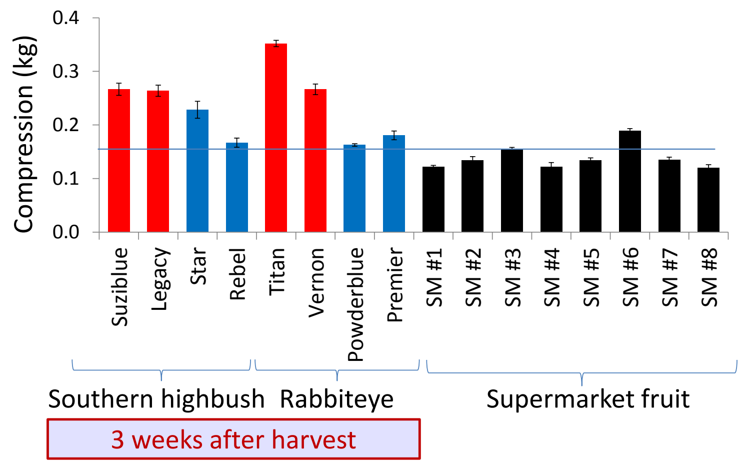

Figure1: Fruit compression values between southern highbush and rabbit eye cultivars (3 weeks after harvest) in comparison to fruit from 8 supermarket (SM) stores. Horizontal blue line is the average compression values of all supermarket fruit.

Fruits are intensive reservoirs of metabolites such as sugars and organic acids. During fruit ripening, fruit undergo many physiological changes such as fruit softening, decrease in acidity, increase in sugars and anthocyanins and changes in aroma. These changes eventually determine the quality, texture, flavor and nutrition during harvest. Some of these metabolite changes continue during postharvest storage. If we can identify specific sugars and acids in ripe fruit that predict fruit quality during extended storage, we can use these metabolites as markers to produce fruit that can maintain quality during extended storage. This approach has been applied to other fruits such as tomato (Carrari et al., 2006; Gómez-Romero et al., 2010), peach (Lombardo et al., 2011), grapes (Degu et al., 2014), and strawberry (Zhang et al., 2011). Metabolite analyses in these fruits have led to the identification of antioxidant phenolic compounds, and flavor components which can eventually lead to improved agronomic traits. In blueberry, identifying compounds that accumulate during ripening and storage will in the future prove extremely critical in extending shelf-life, and in maintaining superior quality of fruit. Further this analysis can be developed as a tool for breeding to identify berries with certain desired characteristics.

Preliminary Results:
Our preliminary results based on evaluation of a few southern highbush and rabbiteye cultivars in 2015 - 2017 suggest that there are differences in shelf-life and fruit quality attributes among cultivars in both southern highbush and rabbiteye blueberry (Wang, NeSmith and Nambeesan,

2016). We measured fruit firmness (compression test) and skin puncture and other fruit quality attributes such as soluble solids and acidity during postharvest storage.

Shown in Figure 2A and 2B are two southern highbush and two rabbiteye cultivars; Rebel and Premier displayed lower fruit firmness (measured by compression) and had lower shelf life in comparison with Suziblue and Titan which were firmer and displayed better keeping quality. Generally, fruit with lower acids and higher sugars appear to have poor fruit quality and shelf-life. Even though in some cultivars SSC (Soluble Solids Content)/TA (Titratable Acidity) was an indicator of fruit shelf-life, in certain other cultivars SSC/TA did not correlate with postharvest fruit quality. Further we measured individual sugars and acids such as sucrose, fructose, glucose, malic acid, citric acid and quinic acid. Our preliminary data indicate that quinic acid content is higher in Suziblue and Titan compared to Rebel and Premier (Fig. 2C and 2D). Whether quinic acid content correlates with better fruit quality during storage requires further investigation with multiple varieties that differ in storage attributes. Our proposal plans to test if in fact some specific metabolites, such as quinic acid, are different in varieties differing in storage attributes.

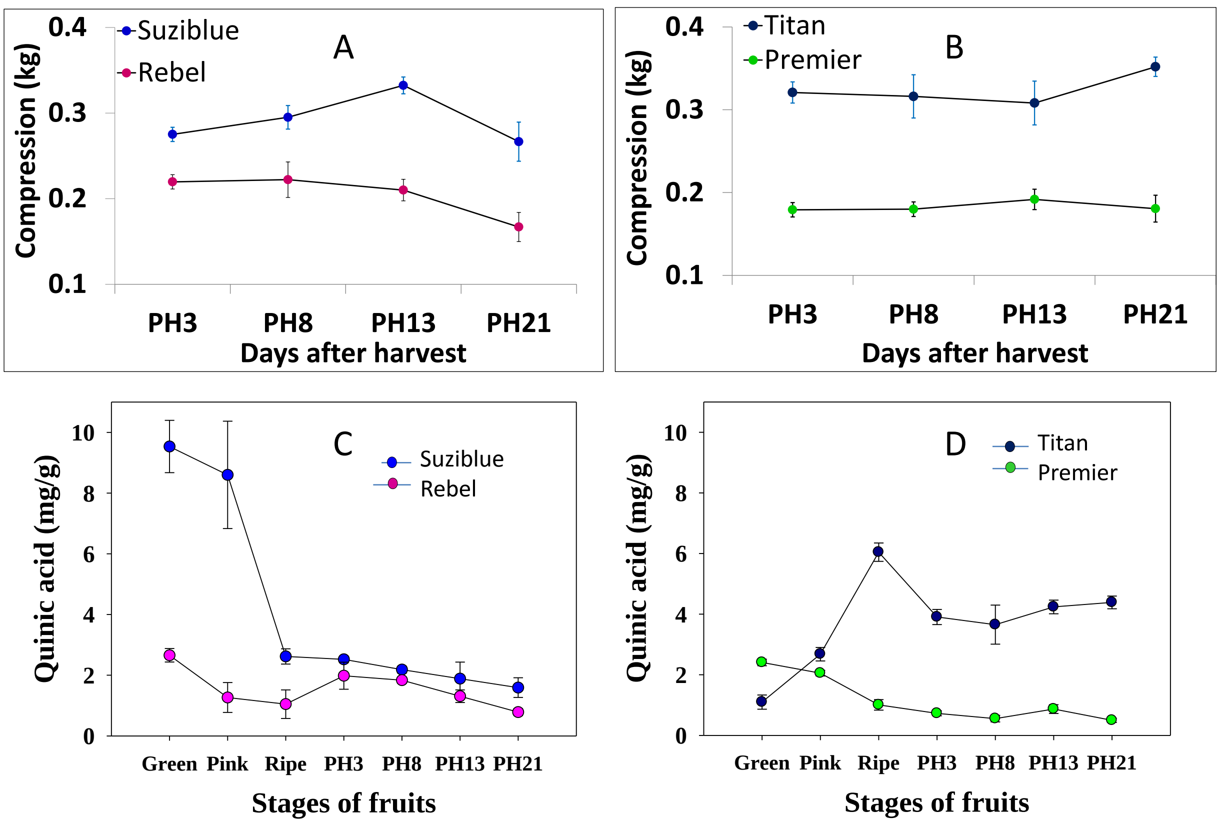

Figure 2. Fruit compression and quinic acid content in (A, C) southern highbush blueberries (B, D) rabbiteye blueberries. Stages of fruit include Green, Pink, Ripe, 3 days after postharvest storage (PH3), PH8, PH13 and PH21.

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. This project would allow for further identification of possible differences within and among older and newer varieties, as well as advanced blueberry breeding selections to be used as possible new cultivars or as parents in breeding programs. Additionally, increased knowledge of changes in sugars and acids in varieties that differ in fruit quality would help to use metabolites as markers that can predict blueberry quality. Overall, knowledge from this project would benefit blueberry growers, consumers, and the industry by providing material with increased quality.

Description of Procedures:
In 2018, 3 southern highbush cultivars, Emerald, Miss Alice Mae, and Rebel were collected at Cornelius Farms in Manor, GA. Three more southern highbush cultivars, Miss Jackie, Miss Lilly, Suziblue, and 4 rabbiteye cultivars, Alapaha, Brightwell, Krewer, Titan, were collected at UGA Blueberry Research Farm in Alapaha, GA. All fruit were harvested at fully ripe stage with four replications. Genotypes selected represented a range of fruit ripening times throughout the season (early, middle and late season ripening). Fruit from all varieties were harvested when plants were approximately 40 to 60% ripe. After harvest, fruit for each genotype was placed in clamshells and transported back to the UGA, Athens campus. Fresh fruit was held at approximately 4ºC under high humidity until all tests are completed.

Fruit Quality Measurement
Fruit quality was determined on a weekly basis for three weeks. For determining fruit quality, weight, firmness, skin puncture, titratable acidity (TA), total soluble solids (TSS) was measured. Briefly, compression and skin puncture measurements was performed using a Fruit Texture Analyzer (Model GS-15, Güss Manufacturing (Pty) Ltd., Strand, South Africa). For measuring TA and TSS, juice from 40 g of fruit was extracted using a blender and centrifuged using a bench top micro-centrifuge. The supernatant was used to determine TSS using a digital handheld refractometer (Atago USA, Bellevue, WA). To determine TA, the supernatant was titrated using automatic mini titrator (Hanna Instruments, Woonsocket, RI). The above parameters will provide valuable information on changes in critical shelf-life and fruit quality characteristics over an extended period of storage.

Sugars and Acids Profiling
For this purpose, nonvolatile acids and sugars from blueberries will be analyzed by gas chromatography using a modification of the procedure previously described by Chapman and Horvat (Chapman Jr and Horvat 1989). Fruit samples will be weighed (~50 mg), ground to a fine powder with liquid nitrogen and extracted using 80% methanol. The extract will be dried under a flow of nitrogen, and re-suspended in Hydroxylamine-HCl. After addition of N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA), samples will be analyzed using a gas chromatograph equipped with a flame ionization detector (GC-2014, Shimadzu, Kyoto). All analyses will be performed using at least three replicates for a given cultivar at a given time of fruit storage. Malic acid, citric acid, quinic acid, fructose, glucose, and sucrose contents will be evaluated.

In 2018, due to a lot of rain, fruit quality was poor in comparison to previous years. In addition, due to unpredictability of weather (rainfall) fruit were collected initially at Cornelius farms and then at the Alapaha research farm. Sugar and acid profiling require extensive work and using fruit from 2018 will not be informative since poor fruit quality and different management practices of the two farms will make data interpretation less meaningful. Thus work is in progress to perform sugar and acid analyses from 2017 fruit. Currently the gas chromatography instrument is under repair and we plan to run samples once the instrument is functional.

Results:

Fruit Quality Measurement

Table 1: Fruit quality attributes including compression, puncture, % defect free fruit, weight, total soluble solids (TSS) and titratable acidity (TA) during postharvest storage at 2-3 days (d), 11-12 d and 20-21 d in southern highbush (SHB) cultivars. Darker shade of orange represent high values and green represent lower values and lighter shade or orange are in-between values. A one way Anova was performed to determine differences between cultivars using JMP Pro 12 (SAS Institute, Cary, NC). Means were separated using Tukey's Honest Significant Difference (HSD) test (α = 0.05).

SHB	Compression				Puncture				% Defect free			
Cultivar	2-3 d	11-12 d	20-21 d		2-3 d	11-12 d	20-21 d		2-3 d	11-12 d	20-21 d	
Miss Alice Mae	0.27	0.27	0.25	A	0.16	0.19	0.16	A	98	96	80	B
Emerald	0.25	0.26	0.27	AB	0.15	0.17	0.16	A	99	97	94	B
Rebel	0.24	0.26	0.23	BC	0.16	0.16	0.15	A	100	99	98	A
Miss Lilly	0.23	0.25	0.21	C	0.16	0.16	0.15	A	97	84	68	C
Miss Jackie	0.18	0.20	0.16	D	0.17	0.16	0.14	A	97	78	44	C

SHB	Weight				TSS				TA			
Cultivar	2-3 d	11-12 d	20-21 d		2-3 d	11-12 d	20-21 d		2-3 d	11-12 d	20-21 d	
Miss Alice Mae	2.1	2.1	2.1	B	13.1	12.8	12.2	B	0.39	0.22	0.19	BC
Emerald	2.2	2.1	2.1	B	13.4	13.5	12.9	AB	0.80	0.58	0.57	A
Rebel	1.9	1.6	1.5	C	14.7	14.6	14.0	A	0.35	0.24	0.15	BC
Miss Lilly	2.6	2.5	2.3	A	13.9	15.1	14.1	AB	0.21	0.25	0.20	C
Miss Jackie	2.0	2.1	2.0	B	15.0	14.1	14.3	A	0.34	0.30	0.31	B

Rebel and Emerald in 2018 were collected from Cornelius farm, and even though these two cultivars do not have very high compression and puncture (similar to 2017) they have good-postharvest shelf-life. This is probably due to the good management practices at Cornelius farms to enhance fruit quality. Further Emerald exhibited high TA in 2017 and 2018.

Miss Alice Mae in both 2018 and 2017 had higher compression and even though puncture values are not the highest they were consistent over two years (2017 and 2018). In 2018, Alice Mae had relatively high postharvest shelf-life (defect free fruit); however last year this cultivar did not store well. These are relatively newer plantings in Cornelius farm and therefore fruit are not yet harvested for the market. Thus slightly different management practices for newer plantings may have caused lower defect-free fruit. Overall Miss Alice Mae is a good variety with good characteristics and has potential for improvement during postharvest storage.

Miss Lilly has relatively higher firmness and puncture (consistent in 2017 and 2018); in 2017 Miss Lilly had very good shelf-life (defect free fruit) but in 2018 it did not have an enhanced shelf-life. This year Miss Lilly was picked in Alapaha research farm (compared to last year in Cornelius farms). In 2018 high rainfall during harvest may have contributed to lower defect free fruit. Overall Miss Lilly is a good variety with good characteristics and has potential for improvement during postharvest storage.

Miss Jackie has relatively lower compression (in 2017 and 2018), however puncture was higher in 2017 than 2018. In 2018 shelf-life (defect free fruit) was lower compared to 2017. This year Miss Jackie was picked in Alapaha research farm (compared to last year in Cornelius farms). In

2018 high rainfall during harvest may have contributed to lower defect free fruit. Moreover in 2017 Jackie had higher TA compared to 2018. More data is required to establish the potential of Jackie fruit attributes during storage.

Table 2: Fruit quality attributes including compression, puncture, % defect free fruit, weight, total soluble solids (TSS) and titratable acidity (TA) during postharvest storage at 4 days (d), 12 d and 21 d from rabbiteye (RE) cultivars. Darker shade of orange represent high values and green represent lower values and lighter shade or orange are in-between values. A one way Anova was performed to determine differences between cultivars using JMP Pro 12 (SAS Institute, Cary, NC). Means were separated using Tukey's Honest Significant Difference (HSD) test (α = 0.05).

RE	Compression				Puncture				% Defect free			
Cultivar	4 d	12 d	21 d		4 d	12 d	21 d		4 d	12 d	21 d	
Alapaha	0.18	0.18	0.19	B	0.12	0.09	0.12	B	87	82	71	B
Brightwell	0.23	0.22	0.22	A	0.12	0.13	0.14	A	95	97	88	A
Krewer	0.17	0.18	0.17	B	0.11	0.13	0.12	AB	89	90	57	B
Titan	0.23	0.23	0.18	A	0.12	0.12	0.14	AB	87	89	58	B

RE	Weight				TSS				TA			
Cultivar	4 d	12 d	21 d		4 d	12 d	21 d		4 d	12 d	21 d	
Alapaha	1.4	1.4	1.3	B	15.6	15.6	15.4	A	0.21	0.17	0.17	D
Brightwell	1.5	1.5	1.4	B	15.4	15.3	15.2	A	0.29	0.24	0.23	B
Krewer	3.0	2.9	3.0	A	14.8	15.1	15.0	A	0.24	0.21	0.20	C
Titan	3.1	2.8	2.8	A	13.2	12.7	12.2	B	0.33	0.36	0.33	A

Brightwell had high compression and puncture in 2017 and 2018 and a long shelf-life (% defect free fruit). However weight of fruit was comparatively lower.

Titan is a big-sized fruit. Titan has high compression and overall good fruit characteristics but it does not store very well over time. This year the fruit also showed cracking, may be due to higher rainfall in summer.

Krewer is another big-sized fruit, however has lower compression and overall does not store very well.

Alapaha has relatively lower compression and may have the potential to store well, however fruit size is small and has relatively lower TA.

When one way Anova was performed to determine differences between various time points during storage for fruit quality, percent defect-free fruit decreased from 3 to 12 to 21 days in southern highbush and from 12 to 21 days in rabbiteye cultivars. TA decreased from 3 to 21 days among both southern highbush and rabbiteye cultivars. There was a decrease in weight between 3 and 21 days among southern highbush but not rabbiteye cultivars.

Sugars and Acids Profiling

As mentioned in the methods, current work is in progress to measure specific sugars and acids using gas chromatography from selected southern highbush and rabbiteye cultivars from 2017. Cultivars evaluated would include Suziblue, Farthing, Emerald, Rebel, Titan, Brightwell, and

Alapaha at various ripening stages; green, pink and ripe and postharvest stages at 3 days after storage and 12 days after storage. Depending on the results more cultivars may be evaluated.

Conclusions:

Rebel and Emerald although have lower firmness, with proper management can have good shelf-life. The performances of these cultivars during shipping will need further evaluations. Among the newer varieties, Miss Alice Mae is firm and has good shelf-life. Miss Lilly is a big-sized fruit, and has good shelf life. Among the rabbiteye cultivars, Brightwell although small has good shelf-life. Titan is a big-size fruit, in 2018 due to rainfall displayed fruit cracking and future work with studying certain management strategy may be important to maintain fruit quality after harvest.

Impact Statement:

Along with data from previous years, this research evaluated shelf-life and fruit quality attributes during postharvest storage for several southern highbush and rabbiteye cultivars. This study will provide valuable information to growers, packers and distributors so that they can make informed decisions on how to handle cultivars that differ in postharvest storage to be able to deliver higher quality fruit to consumers. Future work with sugar and acid profiling may provide information on predictors of fruit quality that could be implemented into breeding programs.

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