Title of Project
Identifying Marketable Attributes in Arkansas Fresh-Market Blackberries

Final Report

Grant Code
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Research Proposal

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Objectives
1. Determine compositional attributes of fresh-market blackberry genotypes from the Arkansas blackberry breeding program
2. Identify nutraceutical attributes of fresh-market blackberry genotypes from the Arkansas blackberry breeding program

Justification and Description
Blackberry (*Rubus subgenus Rubus*) is one of the best examples of a wild-harvested specialty crop that moved to commercial use through breeding efforts. This nutraceutical-rich, fresh-market fruit has the potential for an increased role in commercial markets due to consumers' increasing demand for food products with high functional/health properties. Public and private blackberry breeding programs play a critical role in the future of the blackberry industry. In the South the largest blackberry breeding effort is conducted at the University of Arkansas, Fayetteville (directed by John R. Clark) The Arkansas program contributes the majority of the varieties for the South and is the primary program to help in the southern blackberry industry development. Breeding for enhanced nutraceutical composition of blackberries has been hampered by lack of information on genetic markers that influence genetic and environmental controls and the lack of published information on nutraceutical composition of blackberries. The South has an underutilized capability for the production of fresh-market blackberries and there is a need to identify composition-and nutraceutical-based marketable attributes of fresh-market blackberry genotypes from the University of Arkansas black berry breeding program. As with all crops, breeding and release of new cultivars to address evolving changes and production challenges is vital to keep healthy markets. Data generated from the proposed work will also provide information used in marketing these genotypes.

Methodology:

The composition and nutraceutical attributes of fresh-market blackberries were addressed. Fruit was harvested at the University of Arkansas Fruit Research Station, Clarksville in June 2014. Four cultivars ('Natchez', 'Osage', 'Ouachita', and 'PrimeArk® 45') and 25 advanced selections were harvested. After harvest, the fruit was taken to the Department of Food Science, University of Arkansas, Fayetteville for evaluation of composition and nutraceutical attributes. The experiment was designed as a completely randomized design. The composition and nutraceutical attributes were or will be evaluated with three replicated samples for each genotype. Analyses will be conducted using JMP® (version 11.0; SAS Institute Inc., Cary, NC). Tukey's HSD (Honestly Significant Difference) will be used for mean separation. Pearson's correlation will be used to test the relationship between/within attributes.

Methods for composition analysis (completed June-October 2014)

Three samples of approximately 100 g of berries were collected for each cultivar or genotype, placed in plastic storage bags, and stored at -20°C until analysis. From the frozen berries, three berries per genotype and replication were used to determine berry attributes (individual berry weight, berry length, and berry width) and pyrene attributes (number/berry and dry weight/berry). Three replicate three-berry samples of each cultivar and genotype were used to determine soluble solids, pH, and titratable acidity for each genotype. Samples were thawed, placed in cheesecloth and squeezed to extract the juice from the berries. Titratable acidity and pH were measured by an 877 Titrino Plus (Metrohm AG, Herisau, Switzerland) standardized to pH 2.0, 4.0, 7.0 and 10.0 buffers. Titratable acidity was determined using 6 g of juice diluted with 50 mL of deionized, degassed water by titration with 0.1 N sodium hydroxide to an endpoint of pH 8.2; results were expressed as percent citric acid. Total soluble solids (expressed as percent) was measured with a Bausch & Lomb Abbe Mark II refractometer (Scientific Instrument, Keene, NH).

Methods for nutraceutical analysis (completed February 2015)

To obtain sample extracts, samples (25 g) were homogenized with 20 mL of acetone/water/acetic (70:29.5:0.5 v/v/v) with a Euro Turrax T18 Tissuemizer. The samples were filtered through Miracloth, the filter cakes were isolated, and the extraction was repeated. The filtrates were adjusted to a final volume of 250 mL with extraction solvent. Samples were analyzed by HPLC for ellagitannins and flavonols, total anthocyanins, total phenolics. Oxygen Radical Absorbance Capacity (ORAC) values were determined on a dual pump BMG Fluostar Optima plate reader.

Results:

Twenty nine blackberry genotypes were evaluated for composition and nutraceutical content. Blackberries were harvested from the University of Arkansas Fruit Research Station, Clarksville. The blackberries were harvested at the shiny-black stage of ripeness and were free of major blemishes, flaws or damage. Fruit were hand-harvested from the plants in June-July 2014. Approximately 4 kg of fruit was harvested from each of the genotypes. Air temperatures were the same for both harvests. The fruit was harvested by 11:00 AM. Fruit was harvested directly

into 240-g (pint), low-profile vented clamshells, placed in chilled coolers and transported to the Department of Food Science, Fayetteville. A 100 g sample of each genotype was frozen (-20 °C) in triplicate for composition and nutraceutical analysis.

The group of genotypes in the study provided a substantial range in variables measured. One of the first focus areas was that of soluble solids and pH/titratable acidity (Table 1), as these play a major role in flavor of blackberries, with the sweeter, lower-acid berries usually preferred by consumers. Selection APF-238 had the highest soluble solids (13.33%) and other values ranged down to A-2418 which had the lowest soluble solids (8.07%). The pH ranged from a high of 3.61 for A-2487 to the low of 3.00 for A-2450. Likewise, the titratable acidity values ranged from 0.64% (A-2252) to 1.47 (A-2419). It was very interesting to note that those berries usually observed to have the more desirable flavor usually had a titratable acidity value under 1.0%. This could be a key indicator in evaluation and breeding for increased flavor and acceptance.

In addition to the soluble solids, pH/titratable acidity, the other physicochemical attributes of blackberries (size, number of drupelets, number of pyrenes) play a key role in acceptance. (Tables 2-3) These other attributes had substantial range in values. 'Natchez' blackberries had the highest berry weight (14.26 g), berry length (43.68 mm), number of drupelets/berry (125.83), and pyrene weight/berry (0.43 g). Whereas, A-2487 had the lowest berry weight (4.90 g), A-2480 had the lowest berry length (21.86 mm), and A-2453 had the lowest number of drupelets/berry (50.22) and the lowest pyrene weight/berry (0.18 g).A-2452 had the highest berry volume (7898.96 mm^3) and A-2480 had the lowest (2159.95 mm^3). Berry width ranged from 26.92 mm for 'Osage' and 19.42 mm for A-2480. The number of pyrenes/berry ranged from 123.33 (APF-266) to 51.00 (A-2453). APF-293 had the highest pyrene weight/berry weight (4.70%) and A-2416 had the lowest (2.52%).

In terms of nutraceuticals, that was a substantial range of values. (Table 4). APF 190 had the lowest total flavonols (7.70 mg/100 g) and total anthocyanins (55.36 mg/100 g). APF 266 had the lowest Total phenolics (422.16 mg/100 g) and ORAC (53.10 µmol/g). 'Ouachita' (20.63 mg/100 g) had the highest total ellagitannins and A-2473 (56.50 mg/100 g) the least. A-2419 had the highest total flavonols (21.26 g/100 g), and A-2435 had the highest Total anthocyanins (322.33 mg/100)g). APF 290 had the highest Total phenolics (791.90 mg/100 g), and AFP 238 had the highest ORAC (151.80 µmol/g).

Conclusion

This research provided insight into composition and nutraceutical attributes of fresh-market blackberries from the University of Arkansas Blackberry Breeding Program. The data was used as a base-line for future research to determine how these attributes were perceived by consumers. Evaluating these attributes of fresh fruit is an important tool that can be used to determine commercial potential for selections and cultivars.

Impact Statement

Results identified blackberry composition and nutraceutical attributes that were used by the University of Arkansas blackberry breeding program during breeding selection process, in addition to valuable data for future research on fresh-market blackberries.

Citations for any Publications Arising from the Project

Papers Published

Threlfall, R.T., O.S. Hines, J.R. Clark, L.R. Howard, C.R. Brownmiller, D.M. Segantini and L.J.R. Lawless. 2016. Evaluation of Physiochemical and Sensory Attributes of Fresh Blackberries Grown in the Southeastern United States. HortScience, 51(11): 1351-1362.

Threlfall, R.T., O.S. Hines, and J.R. Clark. 2015. Commercial Attributes of Fresh Blackberries Identified by Sensory Panels. XI International Rubus and Ribes Symposium. June 21-24, 2015, Asheville, NC. International Society for Horticultural Science, Acta Horticulturae, Symposium Proceedings, 1133:391-396.

Abstracts Published

Hines, O. S., J. R. Clark, R. T. Threlfall. 2015. Attributes of Fresh-Market Blackberries Identified by a Trained Descriptive Panel. HortScience 49(9) (Supplement) – 2015 SR-ASHS Annual Meeting. P. S21.

Hines, O.S., J. R. Clark, R. T. Threlfall. 2015. Sensory Comparison of an Extremely Firm Fresh-market Blackberry Selection to Industry Cultivars. HortScience 49(9) (Supplement) – 2015 SR-ASHS Annual Meeting. P. S51.

Threlfall, R. T., O. Hines, D.M. Segantini, J. Clark. 2015. Aromatic impact of fresh blackberries identified by a descriptive sensory panel. HortScience 50(9) (Supplement) – 2015 ASHS Annual Conference. P. S239.

Poster Presentations

Threlfall, R.T., O.S. Hines, D.M. Segantini*, and J.R. Clark, 2015. Aromatic Impact of Fresh Blackberries Identified by a Descriptive Sensory Panel. American Society for Horticultural Science. New Orleans, LA, August 4-7, 2015.

Hines*, O.S., J. R. Clark, and R. T. Threlfall. 2015. Sensory Comparison of an Extremely Firm Fresh-market Blackberry Selection to Industry Cultivars. Southern Region-American Society for Horticulture Science Annual Meeting. January 30-February 1, Atlanta GA.

Oral Presentations

Threlfall*, R.T. O.S. Hines, and J.R. Clark. 2015. Commercial Attributes of Fresh Blackberries Identified by Sensory Panels. XI International Rubus and Ribes Symposium. June 21-24, 2015, Asheville, NC.

Threlfall*, R.T., O.S. Hines, and J.R. Clark. 2015. Investigating the Sensory Attributes of Blackberries. North American Raspberry and Blackberry Conference, February 24-27, 2015, Fayetteville, AR

Hines*, O. S., J. R. Clark, and R. T. Threlfall. 2015. Attributes of Fresh-Market Blackberries Identified by a Trained Descriptive Panel. Southern Region-American Society for Horticulture Science Annual Meeting. January 30-February 1, Atlanta GA.

Table 1. Composition attributes for blackberry genotypes Clarksville, AR 2014.

Genotype	Soluble solids (%)	pH	Titratable acidity (g/L) [z]
A-2252	9.80 cdef [y]	3.58 ab	0.64 d
A-2312	8.83 ef	3.42 abcd	1.02 abcd
A-2316	9.90 cdef	3.10 bcd	1.24 abcd
A-2416	9.47 def	3.16 abcd	1.01 abcd
A-2418	8.07 f	3.08 cd	1.35 ab
A-2419	10.03 cdef	3.06 cd	1.47 a
A-2428	9.87 cdef	3.18 abcd	0.98 abcd
A-2434	9.73 def	3.05 d	1.16 abcd
A-2435	10.33 bcdef	3.33 abcd	0.82 abcd
A-2444	12.30 abc	3.44 abcd	0.67 d
A-2450	8.93 ef	3.00 d	1.16 abcd
A-2452	10.47 bcdef	3.18 abcd	1.33 abc
A-2453	10.63 bcde	3.37 abcd	0.75 bcd
A-2454	10.20 bcdef	3.29 abcd	0.82 abcd
A-2473	10.90 abcde	3.42 abcd	0.90 abcd
A-2480	12.67 ab	3.53 abc	1.00 abcd
A-2487	11.83 abcd	3.61 a	0.79 bcd
A-2491	10.97abcde	3.20 abcd	0.97 abcd
APF-190	8.97 ef	3.20 abcd	0.92 abcd
APF-238	13.33 a	3.25 abcd	0.78 bcd
APF-266	9.13 ef	3.18 abcd	0.84 abcd
APF-268	9.93 cdef	3.16 abcd	0.88 abcd
APF-290	10.17 bcdef	3.29 abcd	1.21 abcd
APF-293	8.67 ef	3.26 abcd	0.97 abcd
APF-298	11.20 abcde	3.26 abcd	1.08 abcd
Natchez	10.17 bcdef	3.17 abcd	1.03 abcd
Osage	8.90 ef	3.58 ab	0.69 cd
Ouachita	10.60 bcdef	3.43 abcd	0.66 d
Prime-Ark45	9.47 def	3.38 abcd	0.81 bcd

[z] Titratable acidity expressed as citric acid

[y] Genotypes were evaluated in triplicate (n=3). Means with different letter(s) for each attribute are significantly different ($p < 0.05$) using Tukey's HSD

Table 2. Berry attributes for blackberry genotypes Clarksville, AR 2014.

Genotype	Berry weight (g)	Berry length (mm)	Berry width (mm)	Berry volume (mm^3) [z]	Drupelet number/ berry
A-2252	6.00 ghijk [y]	28.82 hij	22.15 cdef	3707.58 cde	61.33 ghi
A-2312	8.61 cdefghi	32.88 cdefgh	23.83 abcde	4901.12 bcde	89.00 bcdefg
A-2316	7.13 efghijk	29.89 defghi	21.11 def	3495.84 cde	89.67 bcdefg
A-2416	11.06 bcd	36.80 abcdefg	24.81 abcd	5955.08 abc	88.55 bcdefg
A-2418	9.66 bcde	32.90 cdefgh	25.31 abcd	5562.13 abcd	84.22 cdefgh
A-2419	6.68 efghijk	31.23 cdefghi	21.30 def	3755.66 cde	97.89 abcde
A-2428	8.51 cdefghi	31.34 cdefghi	22.72 abcdef	4237.00 bcde	68.44 fghi
A-2434	9.03 cdefg	34.46 bcdefgh	24.30 abcde	5402.33 abcd	86.22 cdefgh
A-2435	9.19 cdef	36.87 abcdef	24.62 abcde	5859.70 abc	101.89 abcd
A-2444	9.32 cde	30.86 cdefghi	24.11 abcde	4689.53 bcde	52.89 i
A-2450	9.05 cdef	37.32 abcd	22.16 cdef	4844.27 bcde	92.22 bcdef
A-2452	12.66 ab	41.71 ab	26.79 a	7898.96 a	110.44 abc
A-2453	6.01 ghijk	27.47 hij	24.85 abcd	4441.41 bcde	50.22 i
A-2454	8.07 defghij	29.65 defghij	24.69 abcde	4740.69 bcde	64.33 fghi
A-2473	5.78 hijk	29.01 ghij	23.19 abcdef	4112.70 bcde	73.11 efghi
A-2480	5.23 jk	21.86 j	19.42 f	2159.95 e	67.44 fghi
A-2487	4.90 k	23.58 ij	21.42 def	2833.99 de	59.11 hi
A-2491	9.70 bcde	36.76 abcdefg	23.07 abcdef	5185.11 abcd	81.78 defgh
APF-190	8.45 cdefghi	30.80 cdefghi	22.20 bcdef	4010.05 bcde	76.11 defghi
APF-238	5.64 ijk	27.13 hij	22.73 abcdef	3683.11 cde	53.34 i
APF-266	11.12 bc	36.93 abcde	26.08 abc	6600.57 ab	110.33 abc
APF-268	9.07 cdef	36.97 abcde	24.01 abcde	5608.27 abcd	78.22 defghi
APF-290	6.85 efghijk	29.18 efghij	22.31 bcdef	3821.37 bcde	82.56 cdefgh
APF-293	8.97 cdefg	38.36 abc	23.72 abcdef	5675.13 abc	115.67 ab
APF-298	6.27 fghijk	31.60 cdefgh	20.40 ef	3483.46 cde	82.89 cdefgh
Natchez	14.26 a	43.68 a	25.96 abc	7726.93 a	125.83 a
Osage	7.29 efghijk	27.81 hij	26.92 a	5374.68 abcd	70.22 efghi
Ouachita	8.80 cdefgh	29.08 fghij	26.56 ab	5458.53 abcd	69.78 efghi
Prime-Ark45	7.64 efghijk	33.43 cdefgh	22.71 abcdef	4534.55 bcde	85.78 cdefgh

[z] Volume calculated as a cone

[y] Genotypes were evaluated in triplicate (n=3). Means with different letter(s) for each attribute are significantly different ($p < 0.05$) using Tukey's HSD

Table 3. Pyrene attributes for blackberry genotypes Clarksville, AR 2014.

Genotype	Pyrene weight (g)/ berry	Pyrenes/ berry	Pyrene weight/ berry weight (%)
A-2252	0.19 fgh [z]	58.44 ijk	3.18 bcd
A-2312	0.27 cdefgh	90.33 cdefg	3.26 abcd
A-2316	0.30 bcdef	91.89 bcdefg	4.21 ab
A-2416	0.28 cdefgh	83.22 defghij	2.52 d
A-2418	0.38 abc	94.22 bcdefg	3.90 abcd
A-2419	0.27 cdefgh	106.89 abcde	4.04 abc
A-2428	0.29 cdefgh	69.11 fghijk	3.35 abcd
A-2434	0.36 abcd	88.78 cdefgh	4.03 abc
A-2435	0.25 defgh	96.11 abcdef	2.76 bcd
A-2444	0.24 efgh	54.00 k	2.61 cd
A-2450	0.32 abcde	92.00 bcdefg	3.58 abcd
A-2452	0.40 ab	111.78 abcd	3.19 bcd
A-2453	0.18 h	51.00 k	2.93 bcd
A-2454	0.25 defgh	68.56 fghijk	3.15 bcd
A-2473	0.23 efgh	76.00 fghijk	3.96 abcd
A-2480	0.20 fgh	70.89 fghijk	3.77 abcd
A-2487	0.19 fgh	59.89 hijk	3.92 abcd
A-2491	0.28 cdefgh	84.78 defghij	2.92 bcd
APF-190	0.27 cdefgh	78.44 efghijk	3.20 bcd
APF-238	0.18 gh	55.89 jk	3.20 bcd
APF-266	0.29 bcdefg	123.33 a	2.63 cd
APF-268	0.29 bcdefg	78.22 efghijk	3.21 abcd
APF-290	0.26 defgh	88.22 cdefgh	3.87 abcd
APF-293	0.42 a	120.66 ab	4.70 a
APF-298	0.21 efgh	86.44 cdefghi	3.38 abcd
Natchez	0.43 a	115.00 abc	3.12 bcd
Osage	0.26 defgh	72.33 fghijk	3.62 abcd
Ouachita	0.23 efgh	66.78 ghijk	2.66 cd
Prime-Ark45	0.32 abcde	90.22 cdefg	4.21 ab

[z] Genotypes were evaluated in triplicate (n=3). Means with different letter(s) for each attribute are significantly different ($p < 0.05$) using Tukey's HSD

Table 4. Nutraceutical attributes for blackberry genotypes, Clarksville, AR 2014.

Genotype	Total ellagitannins	Total flavonols	Total anthocyanins	Total phenolics	ORAC[z]
A-2252	27.10 ef[y]	12.16 abc	100.40 ghi	442.76 cd	62.60 lm
A-2312	31.46 cdef	7.73 c	121.53 fghi	573.63 abcd	64.66 klm
A-2316	43.46 abcde	14.60 abc	213.83 bcde	766.83 a	79.30 hijkl
A-2416	41.96 abcde	12.10 abc	191.10 cdefg	603.13 abcd	61.33 lm
A-2418	43.10 abcde	12.76 abc	181.86 cdefgh	555.66 abcd	76.56 ijkl
A-2419	48.53 abcd	21.26 a	160.50 defgh	701.23 abcd	92.66 fghi
A-2428	49.70 abc	20.90 ab	268.66 abc	758.13 a	116.96 bcde
A-2434	45.43 abcde	12.76 abc	206.56 bcdef	603.63 abcd	98.33 efgh
A-2435	43.16 abcde	16.43 abc	322.33 a	734.33 ab	122.16 bcd
A-2444	33.36 bcdef	11.63 abc	295.11 ab	670.10 abcd	107.40 cdef
A-2450	27.36 ef	12.26 abc	199.23 cdef	573.83 abcd	91.33 fghi
A-2452	38.70 abcdef	10.96 abc	202.50 cdef	588.30 abcd	94.96 fghi
A-2453	39.90 abcde	15.20 abc	119.96 fghi	606.33 abcd	85.30 ghij
A-2454	49.80 abc	14.76 abc	93.53 hi	723.96 abc	106.96 cdef
A-2473	56.50 a	17.76 abc	99.56 hi	702.86 abcd	127.56 bc
A-2480	51.96 abc	13.00 abc	103.90 ghi	674.93 abcd	128.93 b
A-2487	30.93 def	14.46 abc	96.93 hi	626.56 abcd	104.90 defg
A-2491	30.20 def	9.33 c	107.70 ghi	518.73 abcd	97.73 efgh
APF-190	33.86 bcdef	7.70 c	55.36 i	463.80 bcd	87.16 fghij
APF-238	38.90 abcdef	14.33 abc	116.30 fghi	609.66 abcd	151.80 a
APF-266	35.13 bcdef	9.90 bc	122.43 fghi	422.16 d	53.10 m
APF-268	33.16 cdef	14.33 abc	151.60 efgh	563.36 abcd	69.96 jklm
APF-290	45.56 abcde	12.40 abc	142.10 efghi	791.90 a	80.20 hijkl
APF-293	39.56 abcde	14.26 abc	121.70 fghi	699.86 abcd	76.00 ijkl
APF-298	42.33 abcde	18.06 abc	140.56 efghi	591.23 abcd	88.36 fghij
Natchez	38.76 abcdef	8.96 c	183.76 cdefgh	528.50 abcd	77.50 hijkl
Osage	29.46 ef	16.10 abc	246.53 abcd	580.96 abcd	117.76 bcde
Ouachita	20.63 f	8.93 c	173.03 defgh	434.20 d	80.33 hijkl
Prime-Ark45	36.86 bcdef	13.30 abc	146.46 efgh	517.20 abcd	73.90 ijklm

[z] Oxygen radical absorbance capacity (ORAC) expressed as µmol Trolox eqv/ g, Total ellagitannins expressed as mg ellagic acid eqv/100 g; Total flavonols expressed as mg rutin eqv/100 g; Total anthocyanins expressed as mg acy/100 g; Total phenolics expressed as mg gallic acid eqv/100 g

[y] Genotypes were evaluated in triplicate (n=3). Means with different letter(s) for each attribute are significantly different ($p < 0.05$) using Tukey's HSD