INFLUENCE OF ENVIRONMENT AND CULTIVAR ON FRUIT QUALITY IN NEWLY ESTABLISHED JUNEBERRY (*AMELANCHIER ALNIFOLIA* (NUTT.) NUTT. EX M. ROEM.) ORCHARDS ON THE FORT BERTHOLD RESERVATION

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ABSTRACT

Juneberry (*Amelanchier alnifolia*) has long been treasured as a native prairie fruit. Historically, the hardy shrub was widely used by many North American Indian tribes. Berries were eaten fresh, steamed, mashed, or dried to a brick-like consistency for reconstitution at a later time. The Fort Berthold Reservation had many wild Juneberry bushes growing along the Missouri River bottom, which were lost when Lake Sakakawea was formed by damming the Missouri River. Three Juneberry cultivars, Honeywood, Martin and Smoky, were planted in 3 locations on the Fort Berthold Reservation in 2004. Fruits collected in 2009 were analyzed for their total phenolic (TP) and total monomeric anthocyanin (TMA) contents. Antioxidant capacity was determined by hydrogen atom transfer (ABTS), ferric reducing antioxidant power (FRAP) and radical scavenging (DPPH) assays. Total phenolic and anthocyanin contents were measured using the Folin-Ciocalteu reagent and the pH differential methods, respectively. Soluble solids were measured by refractometry and titratable acids with NaOH titration. Overall, the phytonutrient contents and antioxidant capacity of the Juneberry fruits were similar to those typically reported for other dark-fleshed small fruits, such as grapes, blackberries and raspberries.

Keywords

Juneberry fruits, phytonutrient content, antioxidant capacity

INTRODUCTION

Juneberry (*Amelanchier alnifolia*) belongs to the family Rosaceae and is native to Western North America (Figure 1). This hardy shrub was a staple food of
many North American Indians and was used in a wide variety of ways. Berries were eaten fresh, steamed, mashed, or dried to a brick-like consistency to be used in the winter (Hartman 2008). Many wild Juneberry bushes grew near the Missouri River bottom at the Fort Berthold Reservation until they were lost due to the formation of Lake Sakakawea when the Missouri River was dammed. Juneberries are still an important source of nutrition on the Fort Berthold Reservation and have also become a potential crop for the region’s developing wine and small fruit industries. Several cultivars have been developed for the fruit industry and most are well adapted to the cold climates and mildly alkaline soils of the northern Great Plains (St. Pierre et al. 2005).

Juneberries are an excellent source of bioactive components such as anthocyanins, flavonols, procyanidins, and phenolic acids (Ozga et al. 2007; Bakowska-Barczak and Kołodziejczyk 2008). These phenolic compounds have attracted much interest due to their antioxidant properties and perceived health benefits, including antimicrobial, anti-inflammatory, and anticarcinogenic activities. They have also been implicated as having insulin secretion ability and neuroprotective effects (Han et al. 2007). This study was designed to determine the nutriceutical content (total phenolic and total monomeric anthocyanin content, as well as antioxidant capacity) of the fruit as a food source for residents of the Fort Berthold Reservation and to measure the variability in factors that influence eating quality and wine production (soluble solids and titratable acid content), as a step in the development of commercial orchards.

Figure 1. Juneberry plants in flower and fruit.
METHODS

**Chemicals and Standards.** 2,2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from EMD Biosciences, Inc. (San Diego, CA). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), and trifluoroacetic acid (TFA) were supplied by Sigma Chemical Co. (St. Louis, MO).

**Planting Sites.** In 2004, three Juneberry cultivars, Honeywood, Martin, and Smoky, were planted in three unique locations on the Fort Berthold Reservation. Two of the sites (War Coulee- WC (47°42’N, 101°50’ W) and White Shield –WS (47°38’ N, 101°50’ W)) were irrigated and the third site at Mandaree (47°41’ N, 102°38’ W) was not irrigated. Fruit production at Mandaree was very limited and inconsistent and was therefore not included in this study. At both WC and WS, the dominant soil series was Williams-Bowden (Brockmann et al. 1979). The two sites differed in that WC was planted on a 3% terraced slope and had no protection from the wind, while WS was planted in a nearly level concave swale and was protected from the wind by a tree belt and housing development (Hartman 2008).

Fruit was collected for the first time in 2009 to evaluate the impact of cultivar and location on fruit quality at WC and WS. Three field samples were made for each cultivar, frozen and brought to Brookings for biochemical analyses.

**Fruit Extraction.** Three replications of frozen Juneberries (100 grams) from each of the 2 field locations (WC and WS) and the 3 cultivars (Martin, Smoky and Honeywood) were pureed in a Waring® blender. Three replicate subsamples of each preparation for each of the tests were placed in 15 ml conical centrifuge tubes and stored at -80°C.

**Total Phenolic Measurement.** Sample TP contents were measured according to Singleton and Rossi (1965) with slight modifications. To determine levels of TP, we added 1 mL of each extract to Folin-Ciocalteu’s phenol reagent and water 1:1:20 (v/v) and incubated for eight minutes followed by the addition of 10 mL of 7% (w/v) sodium carbonate. After 2 h, the absorbance of each was measured at 750 nm. Values of TP were estimated by comparing the absorbance of each with those of a standard response curve generated with gallic acid. Results were expressed as µg gallic acid equivalents on a gram fresh weight basis (GAE/gfw).

**Total Monomeric Anthocyanins.** TMA levels were measured by the pH differential method described by Giusti and Wrolstad (2005). Sample extracts were combined in a 1:20 ratio (v:v) with potassium chloride and with sodium acetate buffers (pH 1.0 and 4.5, respectively) in separate vessels. After an equilibration period (15 min), the raw absorbance of each solution was measured at 520 and 700 nm. A corrected absorbance value was calculated as [(A520–A700) pH 1.0 - (A520–A700) pH 4.5]. The anthocyanin content was calculated using the molar absorptivity (ε) and molecular weights (MW) of cyanidin 3-glucoside (ε = 26,900; MW = 449.2). Results are expressed as µg cyanidin 3-glucoside equivalents (Cy3-GE)/gfw.

**Antioxidant Capacity Analysis.** The antioxidant capacity of Juneberry extracts and standard compounds was determined using the three antioxidant assays: fer-
ric reducing antioxidant power (FRAP), 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and 2,2-diphenyl-1-picrylhydrazyl (DPPH). In brief, FRAP assay was performed as previously described by Benzie and Strain (1999). The analysis was conducted at 25-30°C under pH 3.6 condition with a blank sample in parallel at 593 nm. The ABTS assay was performed according to the modified method of Ozgen et al. (2006). Levels of reduced ABTS reactants were measured at 734 nm. DPPH assay was performed according to the method of Brand-Williams et al. (1995) and the absorbance was determined at 517 nm. All assay reactions were kept in the dark for 60 min prior to measurement of the absorbance using Beckman Coulter DU-650 spectrophotometer. The results were expressed as micromoles of Trolox equivalents (TE) per gram fresh weight of Juneberry extracts.

**Fruit Quality.** Juneberry purees were assayed for soluble solid (SS) contents by refractometry and for levels of titratable acidity (TA) using the methodology of Perkins-Veazie et al. (1996).

**Statistical Analyses.** Means, standard errors, regression equations, and Pearson’s correlation coefficients were calculated using Microsoft Excel (Microsoft International, Redmond, WA) spreadsheet functions. Analyses of variance and multiple comparisons using the Tukey HSD were performed using StatCrunch® Data Analysis on the Web (http://www.statcrunch.com/).

**RESULTS AND DISCUSSION**

This study is a first examination of Juneberry production in newly established orchards on the Fort Berthold Reservation. Cultivation methods, plant attrition and long-term survival have been previously described for these plants (Hartman 2008). The data presented here represent the first harvest of fruits from these orchards and provide an initial look at fruit quality.

![Figure 2. Total Phenolic Content, in gallic acid equivalents (\( \bar{X} \pm SE \)), was determined by the Folin-Ciocalteu method. A) Phenolic content by location, was not significantly different. B) Phenolic content by cultivar were was different, \( F = 4.446, P = 0.031, df = 17 \). HHoney wwood contained higher levels of phenolics that than did Smoky (\( \alpha = 0.05 \), Tukey HSD).](image-url)
Total phenolic content, phenolic composition and antioxidant capacity in fruit have been shown to vary in response to cultivar, abiotic stresses such as temperature, water loss and soils, and harvest year (Wang and Lin 2000; Wang and Zheng 2001; Ozgen et al. 2008). This study examined the impact of cultivar and environment on fruit quality and phenolic antioxidants in Juneberries grown on the Fort Berthold Reservation. Juneberry fruit phenolic content (Figure 2) was not significantly different between WC and WS. Cultivars, however, showed a consistent pattern of phenolic content distribution with Honeywood having a significantly higher phenolic concentration compared to that of Smoky, over both locations ($P = 0.05$). Martin was consistently intermediate in phenolic content at the two sites. Location by cultivar interaction was not significant.

Total anthocyanin content (Figure 3) was measured by the pH differential method. Anthocyanin content is reflective of the stage of ripeness as well as the fruit color of a specific cultivar (Ozgen et al. 2008). At the WC site, all three cultivars had significantly higher anthocyanin content than those harvested at WS. The relatively large SE may reflect the difficulty in harvesting uniformly ripe pomes. Juneberry fruit tends to ripen in an uneven manner that makes harvesting labor intensive (St. Pierre 2006). Selection of more uniformity in maturation is a long-term goal of our research.

Regardless of the unevenness of ripening, Honeywood consistently produced the largest amounts of anthocyanins. Over the two locations the Honeywood fruit contained almost twice as much anthocyanin as did the Smoky cultivars and was significantly different ($P = 0.05$) from both the Martin and Smoky fruit. Although the Martin fruit anthocyanin level appears intermediate between the two other cultivars, it was not significantly different from that of Honeywood or Smoky.

That the fruit at the two locations had similar amounts of total phenolics, but showed significant differences in anthocyanin concentrations, which com-
prise the majority of the phenolic compounds in these fruits (Bakowska-Barczak and Kolodziejczyk 2008; Kraft et al. 2008), suggests that the environment at the two locations had an impact on the non-anthocyanin phenolics. This variation in phenolic composition affects the responses of the three different antioxidant measurements (Ozgen et al. 2008) which can be seen in our results.

The three antioxidant assays (Figure 4) utilize different mechanisms to measure the antioxidant capacity of the fruit. Each method provides a different type of data and use of multiple methods has been recommended to provide a complete evaluation of phenolic antioxidants (Ozgen et al. 2006; 2008). ABTS is driven by a direct hydrogen atom transfer from the antioxidant molecules. FRAP measures the ferric reducing antioxidant power of the antioxidant, and DPPH is a radical scavenger. The antioxidant capacity of Honeywood fruit was significantly greater ($P = 0.05$) than that of Smoky fruit over all locations by both ABTS and FRAP measurements, but there was no difference in antioxidant capacity by location. DPPH analyses indicated a significant difference in antioxidant capacity by location ($P = 0.05$), but there was no difference between cultivars.

Figure 4. Total Antioxidant Capacity ($\bar{X} \pm SE$, n = 3) was measured using three separate methods, ABTS, FRAP and DPPH assays, and expressed at Trolox equivalents. A) Only DPPH showed differences across location, ($F = 19.977$, $P = 0.004$, df = 17). B) ABTS, ($F = 15.012$, $P = 0.0003$, df = 17) and FRAP ($F = 10.369$, $P = 0.0015$, df = 17), showed significant differences between cultivars with Honeywood having higher antioxidant potential compared to Martin and Smoky ($\alpha = 0.05$, Tukey HSD) in both tests. Smoky and Martin were also different ($\alpha = 0.05$, Tukey HSD) in the FRAP assay.
Variation in the relative ranking of the 3 cultivars within a specific assay results from variations in the types of phenolic compounds produced in the fruit. Differences between the DPPH results and those of ABTS and FRAP suggest that the non-anthocyanin phenolic species present in the fruit vary more in response to environment than cultivar, as these types of compounds have previously been implicated in the differences in assay responses (Ozgen et al. 2006).

The quality and flavor of fruits and wines made from them depend upon their acidity and soluble solid (e.g. sugars, organic acids, etc.) content (Kader 1999). A low pH with titratable acidity of 0.6-0.9 % and a Brix of >8 are generally preferred by fresh fruit consumers and wine makers (Gallander 1987; Kader 1999). The analysis of all of the Juneberry fruit grown on the Fort Berthold Reservation demonstrated that at both locations for all three cultivars the acid and Brix were found to be within the recommended parameters.

Titratable acids showed no significant differences by location, but Honeywood fruit contained significantly more acid (\( P = 0.05 \)) than did the Smoky fruit across locations (Figure 5). Fruit at WS had a significantly greater percent soluble solid concentration (\( P = 0.05 \)) than did the fruit from WC. As WS is drier on average than WC, these values may represent a variation in fruit water content (Figure 6).

The consistently high Brix and titratable acid content of Honeywood fruit suggest that it is a better candidate for making wines than is the Smoky cultivar grown in North Dakota. However, all three appear to be of value to winemakers and further study into fruit size and yields needs to be made. As flavor is a personal response, sensory panels will need to be conducted before any final judgments can be made as to the best cultivars to grow for food or wine production. In informal tastings, Smoky was often the favorite choice for eating and overall taste.

Our results show that Juneberry fruit quality was impacted by growing location and cultivar. In general, Honeywood appeared to produce the highest

![Figure 5. Titratable acids (\( \bar{X} \pm SE \)) were measured by NaOH titration and calculated as citric acid equivalents. A) There were no significant differences by location. B) The three cultivars showed significant differences in acidity (\( F = 5.93, P = 0.013, df = 17 \)). Honeywood fruit contained significantly more titratable acid than did the Smoky fruit (\( \alpha = 0.05 \), Tukey HSD).](image-url)
quality fruit, with regard to phenolic content, anthocyanins, antioxidant capacity, soluble solids and titratable acids. The fruit from the Smoky cultivar tended to be lowest in quality when measured by these parameters and the fruit from the Martin cultivar almost always intermediate between the two. Average size of the pomes, their water content and total yields must still be ascertained before we can complete development of our recommendations. However, all three cultivars have proven to be successful choices on the Reservation and each provides a variation in flavor and sensory appeal. All of the Juneberry fruit quality measurements fell within the range of other health-promoting small fruits such as grapes, strawberries and raspberries and show great potential as a new crop for production at Fort Berthold.

ACKNOWLEDGEMENTS

Funding was provided by grants from the South Dakota Department of Agriculture Specialty Crop Block Grants, the USDA-CREES and the SDSU Agricultural Experiment Station.

LITERATURE CITED


