Antioxidant Capacity and Quercetin Levels in Alaska Wild Berries

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ABSTRACT. Alaska wild berries were harvested in 2003 to evaluate levels of antioxidants in non-cultivated fruit. Berries were stored frozen for up to 20 months until analysis for levels of quercetin and hydrophilic oxygen radical absorbance capacity (H-ORAC$_{FL}$). H-ORAC$_{FL}$ levels were highest in lingonberry *Vaccinium vitis-idaea* L.(119-320, average 203 μmol of Trolox Equivalents (TE)/g fresh weight (FW), n = 13). H-ORAC$_{FL}$ levels in other berries from interior Alaska averaged 174 μmol TE/g for highbush cranberry *Viburnum edule* (Michx.) Raf., 107 μmol of TE/g for crowberry *Empetrum nigrum* L., and 77 μmol of TE/g for bog blueberry *Vaccinium uliginosum* L. Quercetin levels ranged from 0.5 to 14.6 μg/g FW in these 4 species from interior Alaska. Lingonberry

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The authors thank the following people who contributed to the success of the project: residents of Fairbanks, Alaska, who donated berry samples from interior Alaska; Alan Kurczynski who donated berry samples from southcentral Alaska; and Dr. Jeff Smeenk for all his help.

This project was supported in part by USDA special grants for New Crops for New Markets, New Crops Opportunities IV, and USDA CSREES award number 2002-45046-01405.

The manuscript publication number is 2005-010 of the UAF Agricultural and Forestry Experiment Station in the School of Natural Resources and Agricultural Sciences.

International Journal of Fruit Science, Vol. 6(1) 2006
Available online at http://ijfs.haworthpress.com

doi:10.1300/J492v06n01_06

and bog blueberry had higher levels of quercetin than most other berries tested. Alaskan wild berries are rich sources of antioxidants. doi:10.1300/J492v06n01_06

KEYWORDS. Bog blueberry, *Vaccinium uliginosum*, lingonberry, *Vaccinium vitus-idaea*, highbush cranberry, *Viburnum edule*, crowberry, *Empetrum nigrum*, ORAC

INTRODUCTION

Recent awareness of the health benefits of blueberries and other highly pigmented berries has stemmed from research documenting the presence of phytochemicals that contribute to antioxidant activity in the human diet (Heinonen et al., 1998; Prior and Cao, 2000; Prior et al., 1998). There are several thousand phytochemicals with phenolic chemistry, including simple phenolic acids, complex flavonoids (such as anthocyanins), and flavonols (like quercetin) (Macheix et al., 1990). Flavonoids in the human diet are associated with reduced risk of heart disease, cancer, and other long-term health problems (Hollman et al., 1996; Smith et al., 2000; Sun et al., 2002; Wolfe et al., 2003). Variation in phenolic structure and glycoside attachment affect the antioxidant capacity of these phytochemicals (Rice-Evans et al., 1996). Combinations of phytochemicals are naturally present in plant tissue, and measuring overall antioxidant activity is an alternative to measuring levels of specific phytochemicals. Antioxidant activity of berry extracts can be measured as their ability to scavenge peroxyl radicals and protect against the oxidation of a fluorescent probe. The change in fluorescence of fluorescein is a measure of the oxygen radical absorbance capacity ($ORAC_{FL}$) where high antioxidant activity results in a small loss of fluorescence compared with a blank with peroxyl radicals and fluorescein (Huang et al., 2002; Ou et al., 2001; Wu et al., 2004a). The hydrophilic $ORAC_{FL}$, or H-$ORAC_{FL}$, is usually greater than 90% of the total $ORAC_{FL}$ in fruits because lipids are a small fraction of fresh fruits (Wu et al., 2004a). For apples, Wu et al. (2004a) reported a range of 22-42 μmol of TE/g with H-$ORAC_{FL}$, and less than 1 μmol of TE/g with lipophilic $ORAC_{FL}$.

Many types of berries are common in Alaska and serve as fruit sources for rural and urban residents. Species of *Vaccinium*, *Rubus*, and *Ribes* are abundant in the flora of circumpolar regions (Hultén, 1968). The summer season is short, and the long daylength of summer is distinctly different from that found at lower latitudes. People commonly harvest blueberry species, lingonberries, highbush cranberries, and other wild berries (Stanek and Butcher, 1998) when the berries are ripe in July, August, and September. Similarly, many wild berries are consumed in Scandinavian countries, where climatic conditions in northern latitudes limit the growth of fruit trees. In Finland, the annual harvest of wild berries was estimated at 22 lb per person, and the majority of the adult population participates in picking wild berries (Himelrick, 2001). Häkkinen et al. (1999) reported that quercetin was present in many berries from Finland. Glycosides of quercetin are found in blueberry (Cho et al., 2004), crowberry, and lingonberry (Häkkinen and Auriola, 1998). The objective of this research was to identify the range of antioxidant capacity and quercetin levels in wild Alaskan berries.

MATERIALS AND METHODS

Berry collection. Samples of berries were harvested by local pickers in 2003 and stored frozen until analysis. Berries were harvested from July through October when color and size indicated the berries were ripe. The berries were collected by local pickers from locations near Fairbanks (latitude 64.8° N) and Anchorage (latitude 61.2° N), cities in interior and southcentral Alaska, respectively. Samples of 35 to 600 g were stored frozen at −20°C for 11-20 months until analysis. Samples were analyzed from 7 species of berries from interior Alaska (Table 1) and 15 species from southcentral Alaska (Table 2). Interior Alaska has a continental climate, with warmer summers and colder winters than the maritime climate of southcentral Alaska. Bog blueberry was the species most commonly collected by local residents. Of 69 samples from interior Alaska, 34 were bog blueberry and 13 were lingonberry, another species in the Ericaceae. Both species are abundant in the landscape of native vegetation where cool, moist soils limit tree growth.

Antioxidant analysis. The H-ORAC$_{FL}$ was determined using the fluorescent probe, fluorescein, and peroxyl radicals (Ou et al., 2001; Wu et al., 2004a) and was performed at Brunswick laboratories (Wareham, Mass.). The H-ORAC$_{FL}$ quantifies the water-soluble antioxidant capac-

TABLE 1. Range of H-ORAC$_{FL}$ and quercetin levels in berries collected from interior Alaska.

Common name	Species	Sample Size (n)	H-ORAC$_{FL}$ μmol of TE/g FW	Quercetin μg/g FW
Red fruit bearberry	*Arctostaphylos rubra*	3	51-108	0.2-0.4
Crowberry	*Empetrum nigrum*	8	90-124	2.4-5.1
Northern black currant	*Ribes hudsonianum*	2	44-52	1.0-2.3
Red raspberry	*Rubus idaeus*	2	53-58	0.8-3.8
Bog blueberry	*Vaccinium uliginosum*	34	49-124	1.8-12.4
Lingonberry	*Vaccinium vitis-idaea*	13	119-320	2.0-14.6
Highbush cranberry	*Viburnum edule*	7	145-222	0.5-2.6

TABLE 2. Values of H-ORAC$_{FL}$ and quercetin levels in berries collected from southcentral Alaska.

Common name	Species	H-ORAC$_{FL}$ μmol of TE/g FW	Quercetin μg/g FW
Kinnikinnick berry	*Arctostaphylos uva-ursi*	45, 53	0.5, 0.8
Crowberry	*Empetrum nigrum*	87, 87	3.0, 3.7
Bog cranberry	*Oxycoccus microcarpus*	39, 50	3.9, 4.1
Northern black currant	*Ribes hudsonianum*	63	1.9
Red currant	*Ribes triste*	23	0.5
Nagoonberry	*Rubus arcticus*	51	5.1
Cloudberry	*Rubus chamemorous*	29	0.7
Red raspberry	*Rubus idaeus*	37	0.5
Watermelon berry	*Streptopus amplexifolius*	19	2.1
Alaska blueberry	*Vaccinium alaskensis*	76	0.6
Dwarf blueberry	*Vaccinium caespitosum*	73, 96	2.3, 2.5
Blue huckleberry	*Vaccinium ovalifolium*	111	0.9
Bog blueberry	*Vaccinium uliginosum*	57, 65, 74, 87	2.6, 3.2, 6.0, 26.6
Lingonberry	*Vaccinium vitis-idaea*	171	6.4
Highbush cranberry	*Viburnum edule*	117, 117	0.7, 0.9

ity in units of μmol of Trolox Equivalents (TE)/g fresh weight (FW). Trolox is a water-soluble Vitamin E analog that is used as the calibration standard (Ehlenfeldt and Prior, 2001; Ou et al., 2001). Levels of quercetin were determined by high-performance liquid chromatography (HPLC) after extraction in ethyl acetate and reconstitution in methanol (also performed at Brunswick laboratories). The levels of quercetin in the aglycone form were reported in μg/g FW. Briefly, 2 g of homogenized berry samples were added to 10 mL aqueous 0.3% HCl and 5 mL ethyl acetate, shaken for 1 h and centrifuged for 10 min. The extraction was repeated until no yellow color was visible in the ethyl acetate. The ethyl acetate fractions were dried under N_2 and reconstituted in 5 mL methanol. The HPLC HP 1110 system (Hewlett-Packard, Palo Alto, Calif.) used a diode array detector to measure absorption at 280 and 366 nm. A Phenomenex Luna Phenyl-Hexyl (250 × 4.6 mM) column was used for separation. Mobile phase A (9% acetonitrile and 2% acetic acid aqueous solution) and B (80% acetonitrile aqueous solution) were used with a flow rate of 1 mL/min, at 37°C, and the following proportions of B: 0-10 min, 0%; 10-25 min, 40%; and 25-35 min, 100%.

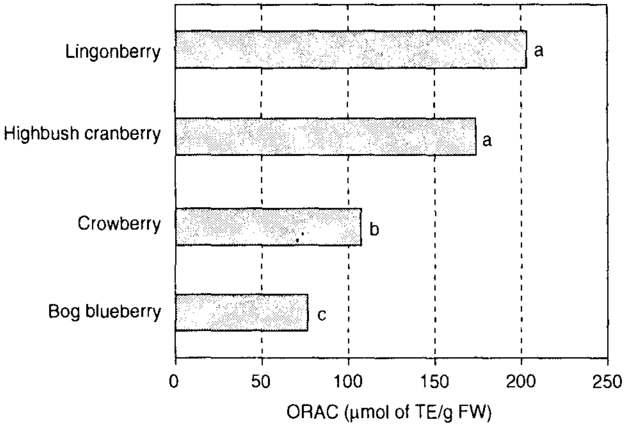
Statistics. The descriptive statistics were calculated using Corel Quattro Pro 10. The data analysis for this paper was performed using SAS/STAT software, Version 9.1 of the SAS System for Windows (Cary, N.C.). Means separation for H-$ORAC_{FL}$ and quercetin levels were calculated when the number of samples was more than four. A one-way ANOVA was calculated using berry type as the independent variable and H-$ORAC_{FL}$ or quercetin level as the dependent variable.

RESULTS

H-$ORAC_{FL}$. In samples from interior Alaska, H-$ORAC_{FL}$ was lowest in Northern black currant and highest in lingonberry (Table 1). In samples from southcentral Alaska, H-$ORAC_{FL}$ was lowest in watermelon berry and highest in lingonberry (Table 2). We found significant differences in H-$ORAC_{FL}$ at $P < 0.001$ among lingonberry, highbush cranberry, crowberry, and bog blueberry from interior Alaska (with $F(3,58) = 56.7$). Mean comparisons (Tukey's) indicate that lingonberry and highbush cranberry were significantly higher than the other berries in H-$ORAC_{FL}$ (Figure 1).

Quercetin. The highest levels of quercetin were measured in lingonberry in both interior and southcentral Alaska (Tables 1 and 2). In all samples from southcentral Alaska, quercetin was present; however, 7 of the 15 species had less than 1 μg/g (Table 2). The highest level (26.6 μg/g) was detected in one sample of bog blueberry from southcentral Alaska, but levels were not consistently high. Lingonberry and bog blueberry had similar levels of quercetin, but lingonberry had H-$ORAC_{FL}$ that was significantly higher than bog blueberry. Highbush cranberry

FIGURE 1. Average values of H-ORAC$_{FL}$ for four species of wild berries from interior Alaska. Bars followed by the same letter are not different ($P < 0.05$) for total H-ORAC$_{FL}$ (μmol of TE/g FW).



and crowberry did not differ in levels of quercetin, but highbush cranberry had H-ORAC$_{FL}$ that was significantly higher than crowberry.

DISCUSSION

Blueberries. The high values for H-ORAC$_{FL}$ in blueberries is consistent with other reports about antioxidant activity in blueberry species. In this study, bog blueberry had 77 Fmol of TE/g, which is similar to 62 and 92 μmol of TE/g for cultivated and lowbush blueberry, respectively, reported by Wu et al. (2004a). Cho et al. (2004) reported a range of 52-139 μmol of TE/g FW for five genotypes of cultivated blueberry. For perspective, Wu et al. (2004a) reported 28 μmol of TE/g for 'Gala' apples and 10 μmol of TE/g for white potatoes.

The wide range reported in antioxidant capacity for blueberries may be related to analysis method. Wu et al. (2004a) and Cho et al. (2004) used fluorescein as the fluorescent probe in ORAC, whereas earlier studies used phycoerythrin. Ou et al. (2001) reported a 3.5-fold increase in ORAC for the antioxidant activity of pure quercetin when fluorescein was compared with phycoerythrin as the fluorescent probe. With phycoerythrin, Kalt et al. (1999) reported 60 and 64 μmol of TE/g FW for highbush blueberry and lowbush blueberry, respectively. Other researchers reported lower values when using phycoerythrin as the fluorescent probe. Ehlenfeldt and Prior (2001) reported 16 μmol of TE/g FW, with a range of 5-31 among 87 cultivars of highbush blueberry. Prior et al. (1998) reported 24 μmol of TE/g FW, with a range of 17-37 among 8 cultivars of northern highbush blueberry. Since different methods of analysis can greatly affect values for ORAC, direct comparisons with earlier studies is difficult.

Fruit size is also a factor in antioxidant capacity of blueberries (Cho et al., 2004; Ehlenfeldt and Prior, 2001). Breeding programs often select large size in cultivated fruit. Wild berries are likely to be small in size and high in phenolics compared with cultivated berries because fertilizer and protection against pests are not commonly applied (Kähkönen et al., 2001). High concentrations of anthocyanins are concentrated near the skin, and smaller blueberries have more skin surface area per unit weight (Ehlenfeldt and Prior, 2001). A similar trend is found in apples, where the apple peels discarded during applesauce processing were found to be a potent source of antioxidant activity (Wolfe et al., 2003). Also, the antioxidant activity in tomato was correlated with smaller fruit size (Hanson et al., 2004).

Other berries. Since H-ORAC$_{FL}$ values for Alaskan blueberries can be considered high at 77 μmol of TE/g, higher values for lingonberry, highbush cranberry and crowberry are noteworthy, at 203, 174, and 107 μmol of TE/g, respectively (Figure 1). This is the first report of high H-ORAC$_{FL}$ in highbush cranberry and crowberry. Earlier studies at our lab indicated high levels of caffeic acid in highbush cranberry (Leiner et al., 2004). For lingonberry, other reports mention high levels of phytochemicals with antioxidant activity (Häkkinen et al., 1999, 2000; Zheng and Wang, 2003). Other berries had H-ORAC$_{FL}$ values that were similar to previous reports. Black currant had a range of 44-63 μmol of TE/g in three samples of Alaskan berries and a range of 49-101 μmol of TE/g FW reported by Wu et al. (2004b) for six cultivars from England, using fluorescein as the fluorescent probe. Red raspberry had a range of 37-58 μmol of TE/g in three samples of Alaskan berries and 24 μmol of TE/g FW reported by Wada and Ou (2002) for cultivated berries from Oregon, using phycoerythrin as the fluorescent probe.

Different berries have different levels of antioxidant capacity. While bog blueberry has less antioxidant capacity than lingonberry, the relatively sweeter taste makes it easy to consume a large quantity of berries. Thus, a larger portion size can deliver equivalent benefit in antioxidant activity. Highbush cranberry has a tart or sour taste, and the portion size for this berry as fresh fruit is likely to be smaller than the sweet-tasting red raspberry.

Alaskan wild berries are rich sources of antioxidants, based on high H-ORAC$_{FL}$ values. While these berries have potential as nutraceutical products, large-scale marketing may be limited by hand harvest of the wild berries. Small plant size and rugged terrain make mechanical harvest challenging. The small amounts of Alaskan berries currently available suggest niche markets and premium prices for products.

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