Antioxidant Detection in Berry Samples from Alaska

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Viburnum edule

Introduction

Health benefits from food go beyond calories for energy and essential vitamins and minerals for metabolism. Many berries and vegetables contain phytochemicals that may have antioxidant activity in the human diet. Medical studies show long term benefits for human health when antioxidants in the human diet are associated with decreased risk of cancer and cardiovascular disease (Perkins-Veazie and Collins 2001). This study looks at nine phytochemicals in berries and vegetables harvested in Alaska. Information on type and quantity of phytochemicals may open new crop opportunities for wild berries from Alaska.

Methods

Plant samples were stored frozen until extraction and analysis. In 2002, there were 62 samples collected from 12 genera of berries, listed below.

Genus and species	Common name	# Samples
Vaccinium vitis-idaea	Lingonberry	10
Rosa acicularis	Prickly Rose	7
Streptopus amplexifolius	Watermelon Berry	7
Vaccinium sp.	Blueberry	7
Viburnum edule	High Bush Cranberry	6
Amelanchier alnifolia	Service Berry	4
Ribes sp.	Red Currant	3
other species		18

Other species of berries had only one or two samples each, including *Prunus pennsylvanica*, *Cornus canadensis*, *Rubus* sp., *Arctostaphylos uva-ursi*, *Empetrum nigrum*, *Fragaria* sp., and *Ribes nigrum*.

In addition to berries, samples from five species of baby greens (leaves for salad mix) were analyzed: the Asian green, mizuna (*Brassica rapa* cv. Kyona), red giant mustard (*Brassica juncea*) and kale (*Brassica oleracea* cv. Bona), red Russian kale (*Brassica napus*), and arugula (*Eruca versicaria*).

Plant samples were extracted by blending 10-20g of frozen plant tissue with 5 parts (weight:volume) of 2 mM (milliMolar) aqueous trifluoroacetic acid (TFA). The extracts were centrifuged, and the supernatant was diluted 1:4 with TFA. The solution was filtered (0.2 microns) prior to HPLC analysis.

The method used UV absorbance detection at 280 nm. The mobile phase was a mixture (3.5-14% gradient) of organic solvent (5 parts acetonitrile to 2 parts methanol) and aqueous solvent TFA at a flow rate of 2 ml/min.

A standard solution of nine phytochemicals showed distinct peaks on a chromatogram. The retention time for each standard was used to indicate the presence of these phytochemicals in the plant extracts.

Discussion

This research will continue in 2004 with more plant samples and further method development for detection of other phytochemicals. This project looked at phenolic acids present in plant tissue. Flavonoids are another type of phytochemical present in many species of berries (Guo and others, 1997; Hakkinen and others, 1998). The flavonoid quercetin was detected in many species of berries from Finland (Hakkinen and others, 1999). On the HPLC, quercetin is usually detected at 360 nm, instead of 280 nm used in this study. Future plans include corroboration of these results using HPLC/Mass Spectrometry and further development for detection of additional phytochemicals. More berry samples from diverse locations in Alaska will be analyzed.

References

Guo C, Cao G, Sofic E, Prior RL. 1997. High-Performance Liquid Chromatography coupled with coulometric array detection of electroactive components in fruits and vegetables: relationship to oxygen radical absorbance capacity. J. Agric. Food Chem. 45:1787-1796.

Hakkinen SH, Karenlampi SO, Heinonen IM, Mykkanen HM, Torronen AR. 1999. Content of the flavonols quercetin, myricetin and kaempferol in 25 edible berries. J. Agric. Food Chem. 47:2274-2279.

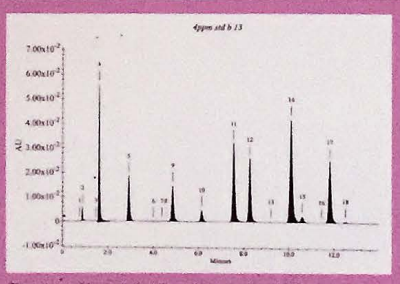
Hakkinen SH, Karenlampi SO, Heinonen IM., Mykkanen HM, Torronen AR. 1998. HPLC Method for screening of flavonoids and phenolic acids in berries. J. Sci. Food Agric. 77:543-551.

Perkins-Veazie P and Collins JK. 2001. Contributions of nonvolatile phytochemicals to nutrition and flavor. HorTechnology 11:539-546.

Results

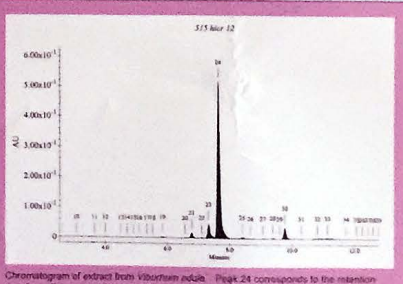
In 2003, the samples of berries and baby greens were extracted during the day and chromatographed overnight. All peak identifications are tentative since they are based on retention times of the standards. Chromatograms from HPLC analysis for all samples were complex in peak size and number. Chromatograms for six extracts of high bush cranberries, Viburnum edule, exhibited intense peaks that indicate the presence of caffeic acid. Chromatograms for seven extracts of rose hips, Rosa acicularis, exhibited peaks that indicate the presence of ascorbic acid. Chromatograms from blueberries and lingonberries had several poorly resolved peaks where a mixture of phytochemicals eluted at similar retention times. When peaks overlapped, quantification of phytochemicals was not precise. Chromatograms from blueberries and lingonberries indicate that the eight phenolic acids studied were present in those berries. Quantification will be part of future research. Gallic acid and p-hydroxybenzoic acid are minor components in the leaves of some species of baby greens.

Rosa acicularis



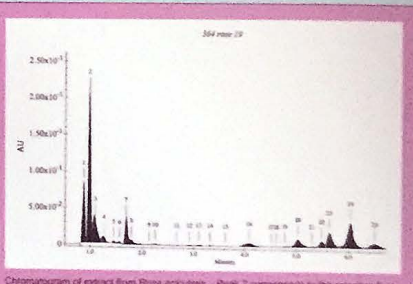
Chromatogram of the standard solution containing ascorbic acid (24 ppm) and eight phenolic acids (4 ppm). Information for each peak number is given below.

Peak #	Phytochemical	Retention Time (min)	Peak Area	Peak Height
2	vitamin C (ascorbic acid)	0.88	17460	5181
4	gallic acid	1.8	238396	56274
5	protocatechuic acid	2.9	112562	19333
9	p-hydroxybenzoic acid	4.85	104285	14671
1	3,4-hydroxyphenyllactic acid	6.13	36424	4717
11	caffeic acid	7.53	247852	31730
12	syringic acid	8.25	167004	25385
14	p-coumaric acid	10.08	361827	41870
17	ferulic acid	11.78	218985	24641



Chromatogram of extract from Viburnum edule. Peak 24 corresponds to the retention time for caffeic acid. Information from six samples is given below. The concentrations (ppm) are measured for the extract solution, which is 1:20th dilution of the berries.

Phytochemical	ppm	average range
vitamin C (ascorbic acid)	22	5-56
gallic acid	<1	
protocatechuic acid	0	
p-hydroxybenzoic acid	<1	
p-hydroxyphenyllactic acid	6	2-24
caffeic acid	52	46-62
syringic acid	2	
p-coumaric acid	<1	
ferulic acid	<1	



Chromatogram of extract from Rosa acicularis. Peak 2 corresponds to the retention time for ascorbic acid, vitamin C. Information from seven samples is given below. The concentrations (ppm) are measured for the extract solution, which is 1:20th dilution of the berries.

Phytochemical	ppm	average range
vitamin C (ascorbic acid)	589	270-888
gallic acid	2	
protocatechuic acid	<1	
p-hydroxybenzoic acid	4	
p-hydroxyphenyllactic acid	14	5-38
caffeic acid	1	
syringic acid	1	
p-coumaric acid	<1	
ferulic acid	<1	

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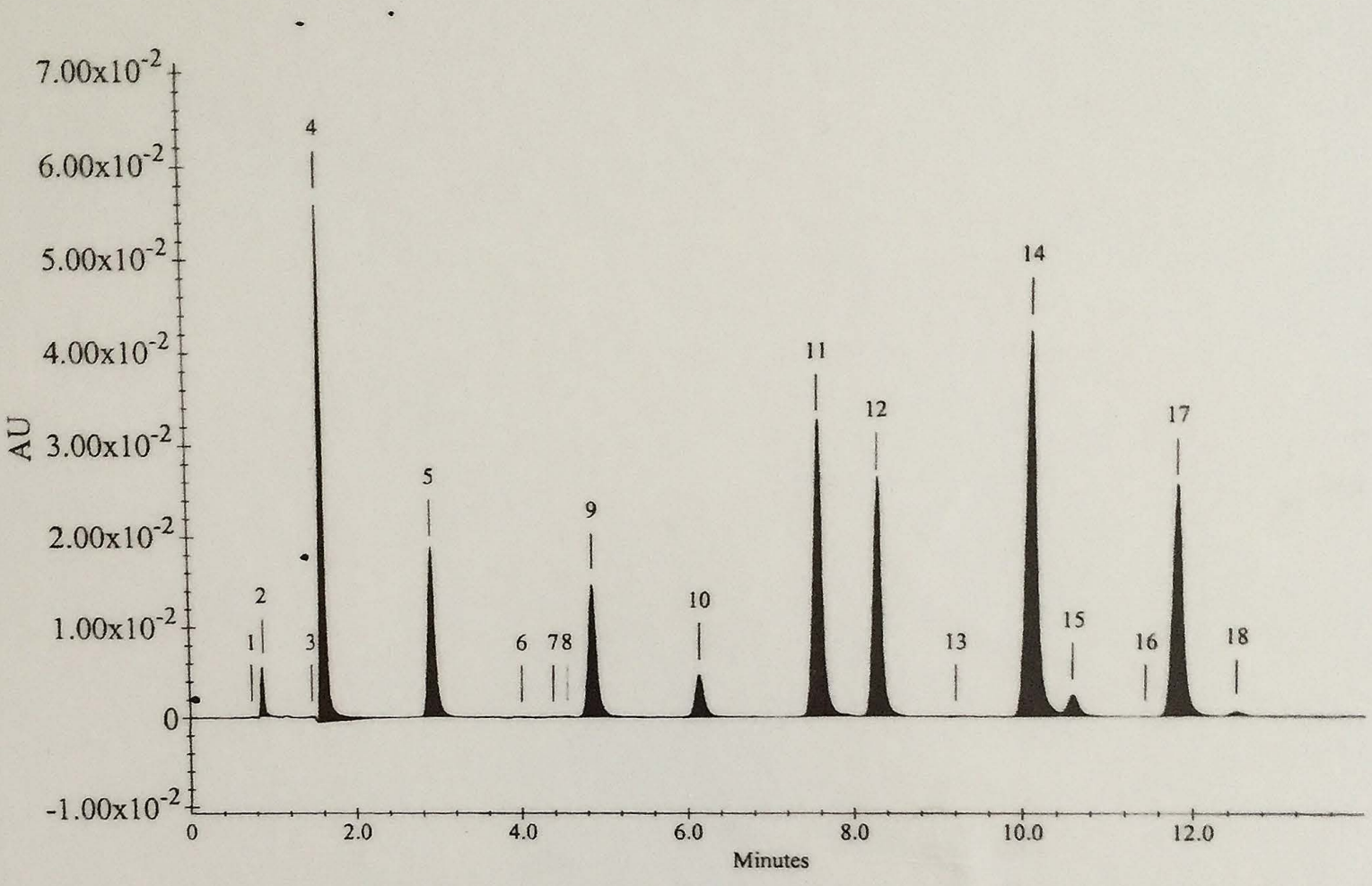

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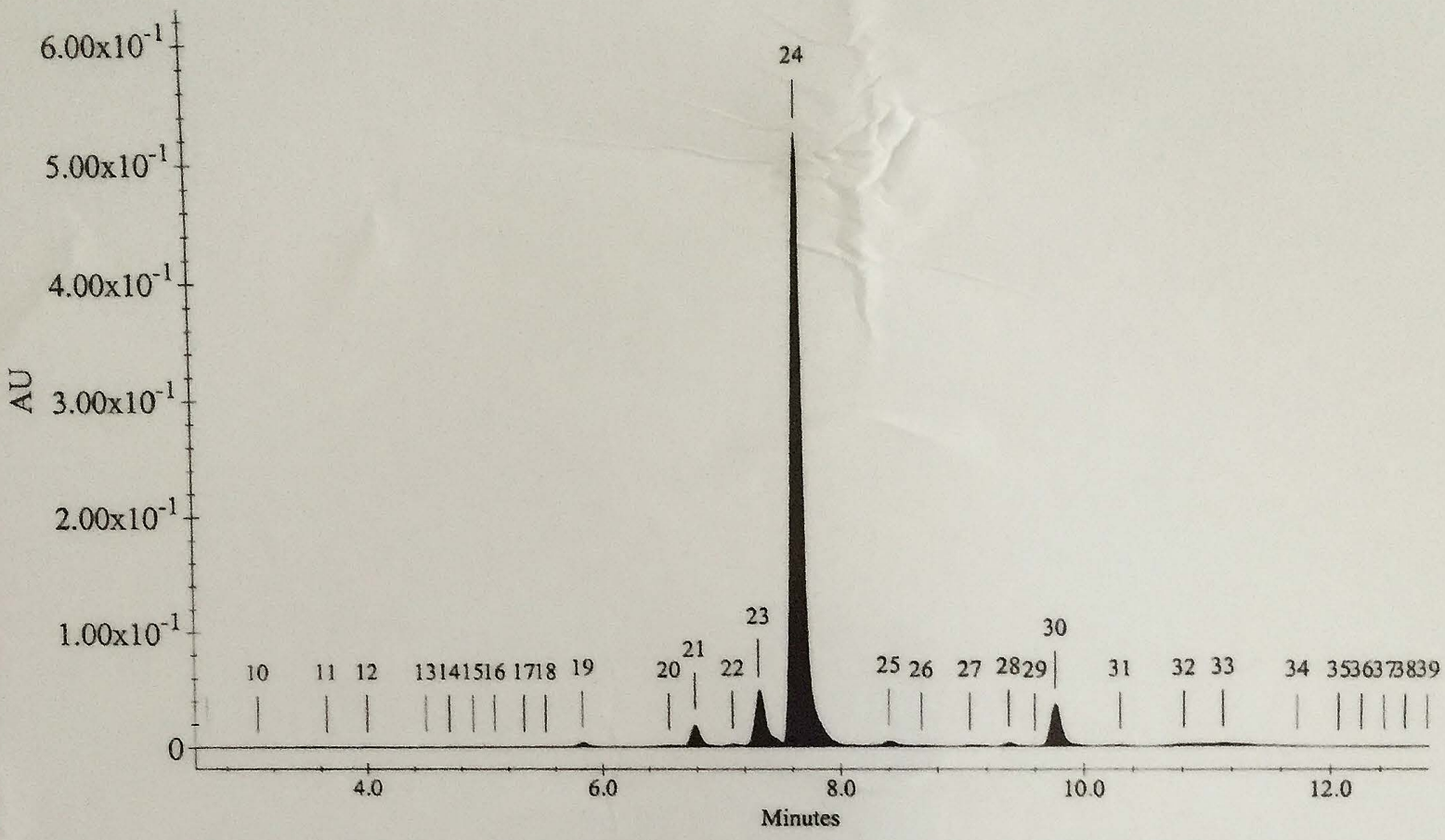

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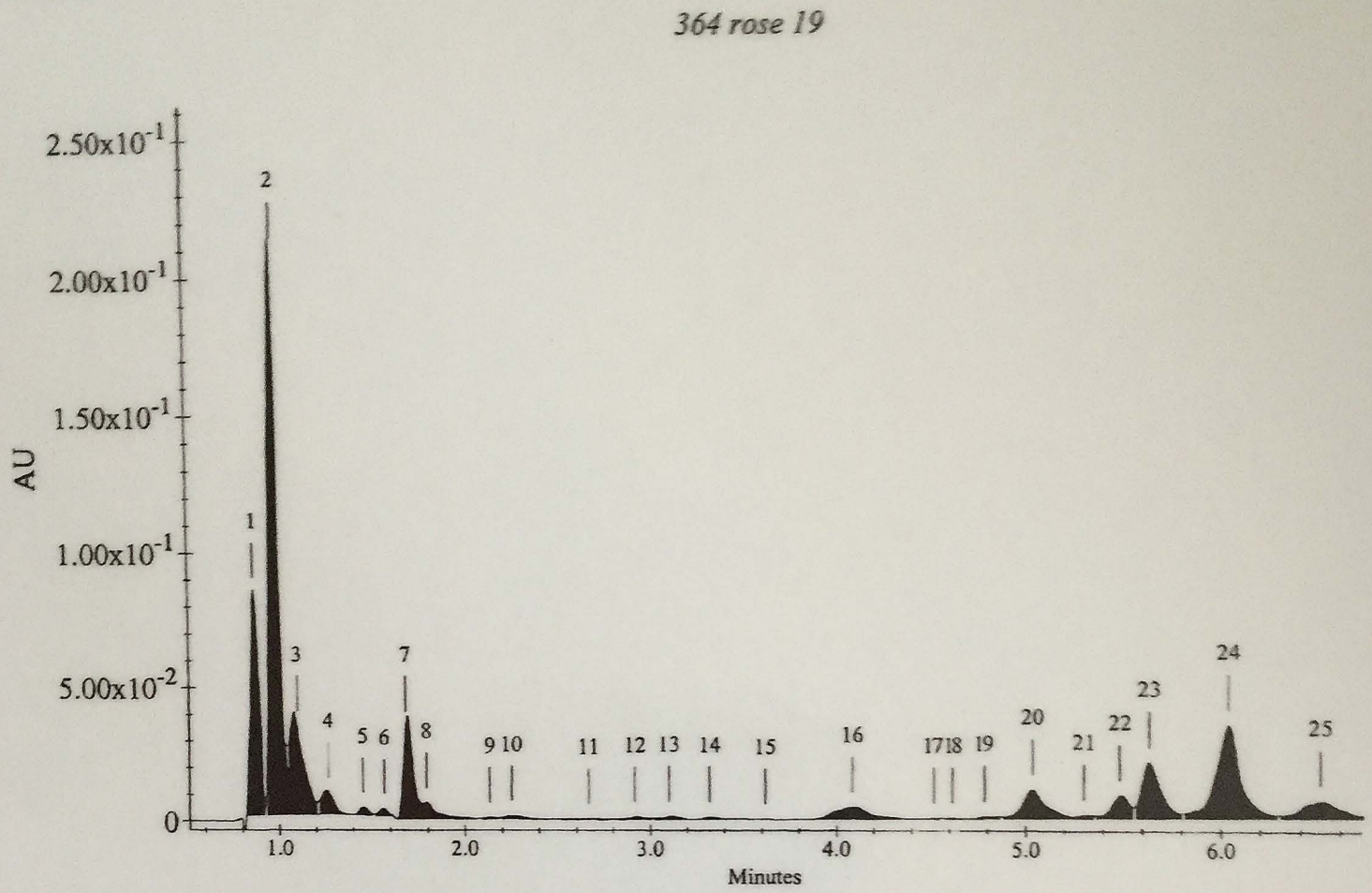
syringic acid	2	
p-coumaric acid	<1	
ferulic acid	<1	



Chromatogram of extract from *Rosa acicularis*. Peak 2 corresponds to the retention time for ascorbic acid, vitamin C. Information from seven samples is given below. The concentrations (ppm) are measured for the extract solution, which is 1/20th dilution of the berries.

Phytochemical	ppm	average range
vitamin C (ascorbic acid)	569	270-888
gallic acid	2	
protocatechuic acid	<1	
p-hydroxybenzoic acid	4	
p-hydroxyphenylacetic acid	14	5-36
caffeic acid	1	
syringic acid	1	
p-coumaric acid	<1	
ferulic acid	<1	