The potential of Manitoba chokecherry as a source of high natural antioxidants

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Consumption of fruits and vegetables is shown to be beneficial for protecting health and preventing some chronic diseases such as cancer, cardiovascular disease, and stroke[1,2]. The positive health effects have been mainly due to the contributions of their natural antioxidant capacity[3]. Chokecherry (*Prunus virginiana*), a unique fruit, is a member of the Rose family and native to North America. Here we demonstrate that chokecherry fruit with strong antioxidant capacity is available in Manitoba, and that its potent antioxidant potential can be developed for health benefits in value-added applications.

Six Manitoba fruits (strawberry, saskatoon berry, raspberry, wild blueberry, seabuckthorn and chokecherry) were selected for comparison of antioxidant properties. All fruit samples were first freeze-dried and then ground to powder prior to analyses. The stone present in fresh seabuckthorn and chokecherry was removed prior to freeze-drying. Their antioxidant components were extracted using ethanol (95%)/1N HCl (85:15, v/v) for analysis of total phenolic content (TPC), total anthocyanin content (TAC), and oxygen radical absorbance capacity (ORAC). Fruit samples were hydrolyzed using 2N NaOH for determination of their phenolic acid composition by HPLC. Quantitative results were expressed on a dry weight basis.

TPCs (equivalents of ferulic acid) were 131.9±8.1, 37.9±1.2, 37.8±1.1, 36.8±1.5, 33.7±1.9 and 22.8±1.4 mg per gram powder for chokecherry, saskatoon berry, wild blueberry, raspberry, strawberry and seabuckthorn, respectively. The TPC of chokecherry was the highest, its TPC level increasing up to three to six times greater than the other fruits. The benefits of phenolic-rich juice from grapes, cherries and berries are to prevent cell death and DNA single-strand breakage against induced oxidative stress through an iron-chelating mechanism[4]. Phenolic compounds in fruits contribute to the *in vitro* inhibition of tumour-cell proliferation[3].

TACs (equivalents of cyanidian-3-glucoside) were 13.1±0.3, 10.8±0.2, 10.0±0.5, 6.6±0.2 and 3.5±0.1 mg per gram powder for chokecherry, saskatoon berry, wild blueberry, raspberry, and strawberry, respectively. TAC in seabuckthorn was not detectable. It was found that chokecherry had a higher TAC than other fruits. The TAC levels in chokecherry were 20 to 270% higher in comparison to the other fruits studied.

Anticancer and antitumour activity of anthocyanins from various sources have been demonstrated, such as inhibition of tumor development and reduction in the proliferation of colon cancer cells[5], prevention of carcinogen-induced colorectal cancer[6], and blocking of breast cell DNA damage[7]. It has also been reported that anthocyanins from chokeberry, grape or bilberry have inhibition effects on the growth of colonic cancer[8].

The total antioxidant capacity was measured by using ORAC assay[9]. Peroxyl radical was generated by 2,2'-azobis (2-methylpropionamide) dihydrochloride (AAPH). ORAC values (equivalent of Trolox) were 3504.7±117.0, 1762.0±116.1, 1731.2±120.5, 1679.8±160.6, 1531.2±84.9 and 1433.3±58.3 mg per gram powder for chokecherry, strawberry, saskatoon berry, wild blueberry, raspberry and seabuckthorn, respectively. Chokecherry had the highest total antioxidant capacity of the Manitoba fruits, its ORAC value being significantly higher by 99 to 145% than the other fruits. . Almost all of the total antioxidant capacity is attributed to the phytochemicals present in fruits, such as phenolic acids, anthocyanins, proanthocyanidins, vitamin C *et al*.

Significant differences in phenolic acid composition and levels were found between chokecherry and other Manitoba fruits (Table 1). Chokecherry showed extremely high levels of caffeic acid, up to

6455.5±502.6 mg per kilogram powder. Caffeic acid levels decreased in the order: saskatoon berry > wild blueberry > red strawberry > raspberry > seabuckthorn. The corresponding values were 2087.8±30.6, 1472.6±5.8, 23.8±2.4, 33.7±4.3 and 9.6±1.7 mg per kilogram powder, respectively. Chokecherry had 2.1, 3.4, 188.9, 268.0 and 644.5 times higher caffeic acid levels than saskatoon berry, wild blueberry, raspberry, strawberry and seabuckthorn, respectively. Chokecherry also contained high *p*-coumaric acid (952.8±42.8 mg/kg). Its *p*-coumaric acid was 3.5, 10.6, 12.2, 13.0 and 23.4 times higher in comparison with the level found in strawberry, saskatoon berry, seabuckthorn, raspberry and wild blueberry, respectively. Other phenolic acids found in chokecherry at low levels were protocatechuic acid (213.8±6.3 mg/kg) and ferulic acid (43.0±4.9 mg/g). The protocatechuic acid content of chokecherry was still 0.6, 1.1, 1.8, 4.1 and 5.3 times higher when compared to that of saskatoon berry, raspberry, wild blueberry, seabuckthorn and red strawberry, respectively. Other significant findings were the high trans-cinnamic acid (566.4±8.6 mg/kg) present in red strawberry and high gallic acid (1129.1±62.4 mg/kg) measured in raspberry in comparison to other fruits.

It is very useful to evaluate phenolic acid composition in plants for application purposes. Caffeic acid has novel and therapeutic effects on hepatocarcinoma cells[10] and protects WI-38 human lung fibroblast cells against H_2O_2 damage[11]. *p*-Coumaric acid protects the heart against doxorubicin-induced oxidative stress[12] and demonstrates good antiplatelet activity for the prevention of vascular disease[13]. Protocatechuic acid has been shown to have the effect of inducing hepatocellular carcinoma cell death[14].

We suggest that chokecherry with its exceptionally high antioxidant capacity is a valuable fruit for potential use as a source of potent natural antioxidants. These findings are useful for developing novel value-added antioxidant products from chokecherry because of its phytochemical profile associated with health protection and prevention of disease. The results also provide evidence essential for breeding novel cultivars of fruit plants with strong natural antioxidants. Research to demonstrate the inhibition effects of phytochemicals from chokecherry on cancer cells *in vitro* is underway.

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Table 1 Phenolic acid composition of Manitoba Fruits

Phenolic acid	CBWS	SKB	WBB	SB	RB	SWS	LSD
GA (mg/kg)	nd	22.3±3.9c	190.0±3.4b	212.4±17.8b	1129.1±62.4a	41.7±9.7c	75.72
PA (mg/kg)	213.8±6.3a	132.0±3.3b	76.4±8.1c	34.0±1.6d	102.0±25.5c	42.3±1.6d	27.78
p-HA (mg/kg)	nd	7.1±3.9c	nd	123.9±4.5a	53.8±8.8b	nd	21.15
VA (mg/kg)	nd	nd	55.8±2.7	nd	nd	nd	---
CA (mg/kg)	6455.5±502.6a	2087.8±30.6b	1472.6±5.8c	23.8±2.4d	33.7±4.3d	9.6±1.7d	503.03
SYA (mg/kg)	nd	nd	286.3±2.5	nd	nd	nd	---
p-CA (mg/kg)	952.8±42.8a	82.1±4.2c	39.3±1.7c	212.8±2.2b	67.5±7.4c	72.4±2.6c	43.52
FA (mg/kg)	43.0±4.9ba	50.2±3.3a	41.3±6.2ba	14.4±1.1c	34.6±5.7b	15.2±1.8c	10.51
SIA (mg/kg)	nd	nd	nd	nd	17.5±2.1	nd	---
o-CA (mg/kg)	nd	nd	nd	16.0±2.0	nd	nd	---
EA (mg/kg)	nd	20.4±5.9b	23.8±4.5b	19.7±9.4b	52.1±8.4a	nd	20.36
trans-CA (mg/kg)	nd	nd	8.7±2.5b	566.4±8.6a	nd	nd	4.07

LSD, least significance difference at $P \leq 0.05$ level of probability. Mean values for samples having similar letters in the same row are not significantly different. GA, gallic acid; PA, protocatechuic acid; *p*-HA, *p*-Hydroxybenzoic acid; VA, vanillic acid; CA, caffeic acid; SYA, syringic acid; *p*-CA, *p*-coumaric acid; FA, ferulic acid; SIA, sinapinic acid; *o*-CA, *o*-coumaric acid; EA, ellagic acid; trans-CA, trans-cinnamic acid. CBWS, chokecherry without stone; SKB, Saskatoon berry; WBB, wild blueberry; SB, strawberry; RB, raspberry; SWS, Seabuckthorn without stone. nd, not detectable.

End notes

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