Purification, composition and antioxidant activity of polysaccharides from wolfberry, cherry, kiwi and cranberry fruits

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Summary

Water-soluble polysaccharides from wolfberry (*Lycium barbarum* L.), sweet cherry (*Prunus avium* L.), kiwi (*Actinidia chinensis* L.) and cranberry fruits (*Vaccinium macrocarpon* Aiton) were extracted with boiling water, fractionated using ion exchange column chromatography, and characterized for molecular weight by high performance size exclusion chromatography (HPSEC). Monomer sugar composition was determined by gas chromatography (GC), and antioxidant activity was assayed by oxygen radical absorbance capacity (ORAC) and Trolox equivalent antioxidant capacity (TEAC). All four types of fruit investigated had four separate polysaccharide fractions; however, the polysaccharides from sweet cherries had higher molecular weight fractions. All the fruits contained rhamnose, fucose, arabinose, xylose, mannose, galactose, and glucose, but the polysaccharides from different fruits, and from cherries of different cultivars and maturity levels, had different ratios of simple sugars. TEAC and ORAC assays revealed that raw and purified polysaccharides from cherries, cranberries, kiwi, and wolfberries have antioxidant activity, and sweet cherry polysaccharides have the highest antioxidant activity.

Keywords: Antioxidant activity, molecular weight, purification, polysaccharides, sugar composition

Introduction

Fruits from the wolfberry (*Lycium chinense* Mill. and *Lycium barbarum* L.) have been consumed for their putative medicinal benefits for thousands of years in China. They are alleged to have various beneficial attributes, including protection of the liver (Gu et al., 2007) and reproductive system (Huang et al., 2004), antioxidative activity (Li et al., 2007) and anticarcinogenic properties (Gan et al., 2004).

In recent years, polysaccharides from food plants have emerged as an important class of bioactive natural products that are being widely studied in order to better understand the relationship between physico-chemical properties and biological activities of these compounds (Inngjerdingen et al., 2005; Gross et al., 2006; Chen et al., 2008; Yuan et al., 2008). Polysaccharides from wolfberry are reported to possess important bioactive functions, including hypoglycemic and hypolipidemic activities (Luo et al., 2004), and immuno-modulating action (Luo et al., 1999). The separation process, structural characterization and biological functions of wolfberry polysaccharides have been extensively investigated over the last several decades. They are thought to be associated with some polypeptides, and polysaccharide–protein complexs (glycoconjugates) are believed to be the important bioactive component of wolfberry (Gan et al., 2003; Gan et al., 2004). Fractions of polysaccharides from wolfberry fruits have been isolated and structurally elucidated (Huang et al., 1999; Peng and Tan, 2001), however, the relationships between their bioactive functions and their chemical composition, as well as the structure of their active components, are not well established.

Numerous studies have suggested that the intake of fruits and vegetables could decrease the risk of coronary heart disease (He et al., 2007; Nikolic et al., 2008) and certain cancers (George et al., 2009). Lipid oxidation may play a role in coronary heart disease, atherosclerosis, cancer and the aging process (Jadhav et al., 1996). There are also a few recent studies showing that purified polysaccharides from wolfberry and other sources exhibit antioxidant activity (Li et al., 2007; Yuan et al., 2008; Ajisaka et al., 2009), but there are no reports on the antioxidant activity of polysaccharides from cherry, cranberry and kiwi fruits. Thus, a goal of our present study was to examine and compare the relative antioxidant activity of polysaccharides from wolfberry, cherry, cranberry

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and kiwi fruits.

Various techniques have been developed to extract wolfberry polysaccharides. The most commonly used method is the hot water extraction process originating from the traditional extraction process for Chinese medicinal herbs (Gan et al., 2003; Gan et al., 2004). Also, there are a few reports on the polysaccharides of kiwi fruits (Sutherland et al.,1999; Ciardiello et al., 2008), cranberry (Popov et al., 2006) and cherry (Thibaul, 1983), however, there are no published reports comparing the concentration, composition and properties of polysaccharides in wolfberry, kiwi, cranberry and cherry. This comparison is aimed at improving our understanding of the compositional and functional aspects of these fruits, which could lead to a better appreciation of these important fruits. Thus, in the present study, water-soluble polysaccharides from wolfberry, sweet cherry, kiwi and cranberry fruits were extracted by a standardized boiling water procedure which has been normally used for the extraction of wolfberry polysaccharides (Li et al., 2007). The extracted polysaccharides were fractionated on a diethylaminoethyl (DEAE) Sepharose ion exchange column chromatography, and characterized for molecular weight by high performance size exclusion chromatography (HPSEC). Monomer sugar composition and antioxidant activity of all extracts and fractions were also determined and compared.

Materials and methods

Materials

Dried wolfberry fruits (*Lycium barbarum* L.) were produced in 2007 in the Zhongning County, Ningxia Hui Autonomous Region, China. After shipping to Agriculture and Agri-Food Canada Research Centre (Summerland, BC, Canada), the dried wolfberry was stored at -30 °C before use. Kiwi fruits (*Actinidia chinensis* L.) were produced at the Agriculture and Agri-Food Canada Research Centre (Agassiz, BC), and kept at -30 °C before use. Cranberries (*Vaccinium macrocarpon* Aiton) were purchased from a local supermarket (Safeway, Penticton, BC, Canada) and stored at -30 °C before use, and sweet cherries (*Prunus avium* L.), including three cultivars (Lapins, Skeena and Sweetheart), and Lapins cherries harvested at three different maturity levels (undermature, mature and overmature) were picked from the orchard of the Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, Summerland, BC. Dialysis tubing Spectra/Por® 7 (Molecular weight cut off = 3,500 Da) was purchased from VWR International Ltd. (Delta, BC, Canada).

Chemicals

Dextran standards with different molecular weights (4,400, 9,900, 21,400, 43,500, 124,000, 196,000, 277,000, 401,000 Da) were purchased from Waters (Milford, MA, USA). Dextran standards with molecular weights of 670,000 Da and 1,400,000 Da were obtained from Sigma – Aldrich (Oakville, Ontario, Canada). Rhamnose, arabinose, xylose, galactose, mannose, glucose and fucose were also purchased from Sigma-Aldrich, while DEAE Sepharose Fast Flow was from GE Healthcare Life Science (Baie-d′Urfé, Quebec, Canada). All other chemicals used were of analytical grade or higher.

Extraction of polysaccharides from fruit

Wolfberry fruits (20 g dry solids equivalent) were soaked in 100 mL distilled water overnight and then blended for 3 min. This was followed by the addition of an additional 100 mL of distilled water to the blended mixture. The mixture was then extracted using continuous stirring on a stir plate for 2 h at 100 ºC (Li et al., 2007). After centrifugation at × 8,627 g for 15 min, the residues were then extracted two more times using the same process. The supernatants were combined and concentrated to about 1/4 volume of the original using a rotary evaporator at 40 ºC. Four volumes of anhydrous ethanol were added to the solution to give an 80 % final concentration of ethanol, and the mixture was kept overnight at 4 ºC to precipitate the polysaccharides. After centrifugation at × 8,627 g for 15 min, the precipitate was washed with 95 % ethanol, 100 % ethanol and acetone three times, respectively (Li et al., 2007). The residues were dried in a vacuum oven at 40 ºC.

Kiwi fruits, which had been kept at -30 ºC for about 6 weeks, were taken out of freezer for 10 min to thaw. Then each kiwi fruit was trimmed to remove the two end parts of the fruit, and cut into 16 small pieces. The chopped fruits were then frozen in liquid nitrogen and kept at -30 ºC for later extraction. For each sample, 20 g dry solids equivalent of chopped kiwi fruit were blended with 200 mL distilled water for 3 min, and then the extraction of polysaccharides from kiwi was similar with that of wolfberry. The extraction of polysaccharides from sweet cherries and cranberries was carried out as described above for kiwi.

Separation and fractionation of polysaccharides

The vacuum oven dried polysaccharide-rich raw extracts (250 mg) prepared as described above were dissolved in 25 mL Milli-Q water at 80 ºC for 2 h

under vigorous stirring, and kept stirring overnight at room temperature to produce about 10 mg/mL polysaccharides solution. The resulting solution was applied onto a DEAE Sepharose Fast Flow packed column (2.5 × 20 cm). The column was eluted at a flow rate of 2.0 mL/min with distilled water and with 0.05, 0.1, 0.25 and 0.5 M NaCl for 2 h, respectively (Huang et al., 1998; Wang et al., 2002; Gan et al., 2004).

Fractions were collected every 5 min and the fractionation procedure was monitored by the phenol-sulfuric acid method (Dubios et al., 1956). Fractions containing carbohydrates were pooled, dialysed in Spectra/Por® dialysis membrane overnight and freeze-dried.

Molecular size distribution of polysaccharides

The molecular weight of the polysaccharide fractions was determined with high performance size exclusion chromatography (HPSEC). The HPSEC system consisted of three columns containing Ultrahydrogel 120, 250 and 2000 (7.8 × 300 mm, Waters, Milford, MA, USA) in series and in combination with a guard column (Ultrahydrogel, 6 × 40 mm, Waters, Milford, MA, USA) to maximize the resolution.

The elution was carried out with 0.15 M NaCl at a flow rate of 0.5 mL/min (Tamaki et al., 2008). The injection volume of standards and samples was 20 μL, and the running time was 90 min. The eluant was monitored using an Agilent 1200 series refractive index detector (Agilent, Santa Clara, California, USA). Retention times from HPSEC were recorded and time variations were dependent on the average molecular weight of the dextran standards. Dextran standards with different molecular weights (4,400, 9,900, 21,400, 43,500, 124,000, 196,000, 277,000 and 401,000 Da) were prepared using the same solvent as the mobile phase. All sample solutions were filtered through a 0.45 μm membrane filter (Chromatographic Specialties Inc., Brockville, Ontario, Canada) prior to analysis. The GPC data analysis software (Agilent, Santa Clara, California, USA) was used for molecular weight data processing.

Sugar composition analysis of polysaccharides

About 5 mg of dialyzed raw polysaccharides sample, and their fractions after DEAE Sepharose Fast Flow column chromatography, were treated with 2 mL 2 M trifluoroacetic acid (TFA) in Teflon-capped, screw-top glass tubes at 100 ºC for 4 h (Goubet et al., 2002) in a heating block of a Reacti-Therm™ Heating/Stirring Module (Model 18790). After hydrolysis, the TFA was evaporated at 50 ºC with a stream of N_2 (Dien et al., 1997) using the Reacti-Vac™. Samples were resuspended in 1 mL deionized water, and neutralized by adding about 0.07 g calcium carbonate to the hydrolysate. The samples were then filtered through a 0.2 μm PVDF filter. Ten μL of 20 mg/mL myo-inositol solution was added to 0.5 mL of filtrated hydrolysate as an internal standard. The neutralized hydrolysate was converted into its alditol acetate derivatives and subjected to GC analysis according to the procedure of Blakeney (1983). The alditol acetate derivative of neutralized hydrolysate was analyzed on an Agilent DB-225 glass-capillary column (30 m × 0.32 mm, i.d., film: 0.25 μm) by gas-liquid chromatography (Mcfeeters et al., 1987) in an Agilent 6890N Network GC System, with a FID detector. Injector and detector temperatures were 225 ºC and 250 ºC, respectively. The oven temperature was held constant at 220 ºC. Helium was used as the carrier gas at a flow rate of 1.5 mL/min. As references, different concentrations of the following neutral sugar standard mixtures were also prepared, converted to their derivatives, and analyzed: rhamnose, fucose, arabinose, xylose, mannose, galactose and glucose.

Antioxidant activity

The antioxidant activity was measured by oxygen radical absorbance capacity ($ORAC_{FL}$) analysis, employing fluorescein as the flurorescent probe according to Ou et al. (2001) procedure. This method measures the ability of antioxidant compounds in tested materials to inhibit the decline of fluorscence which is induced by the peroxyl radical generator 2, 2'-azobis (2-amidinopropane) dihydrochloride (AAPH). The method was modified to adapt to a 96-well fluorescent microplate reader (Molecular Devices, Sunnyvale, CA, Gemini EM) for an excitation wavelength of 485 nm, emission wavelength of 530 nm, and cutoff wavelength of 515 nm. The antioxidant activity of crude polysaccharide extracts and polysaccharide fractions was also measured by Trolox equivalent antioxidant capacity (TEAC) analysis as described by Fukumoto and Mazza (2000).

Other analyses

Total carbohydrates were assayed by phenol-sulfuric acid (Dubios et al., 1956). Uronic acid content was determined colorimetrically by the m-hydrodiphenol test using glucuronic acid as a standard (Blumenkrantz et al., 1973). Moisture content was determined according to the AOAC method (Clark, 2006).

Result and discussion

Extraction and fractionation of polysaccharides

The yields of boiling water extractable crude polysaccharides from wolfberries, kiwi, cranberries, "Sweetheart" cherries, "Lapins" cherries, and "Skeena" cherries were 8.4 %, 9.8 %, 10.6 %, 4.7 %, 5.4 % and 11.0 %, respectively (Table 1). The polysaccharides values for "Lapins" cherries harvested at different maturity levels showed that the concentration of hot water extractable polysaccharides decreased with ripening (data not shown). This reflects the increase of soluble solids content with the increase of maturity level, and indicates that high molecular polysaccharides are converted to low molecular sugars during ripening.

Table 1. Yield, molecular weight and uronic acid of hot water extracted polysaccharides and their fractions from wolfberry, kiwi, cranberry and cherry

Sample	Yield (%)	Molecular weight (kDa)	Uronic acid (%)
Wolfberry			
R-E	8.4±0.1[a]	604/57.6[d]	26.87±2.74[b]
F-2	-	56.6[d]	na
F-3	-	76.2[d]	na
F-4	-	536.7/95.4[d]	36.64±5.42[c]
Kiwi			
R-E	9.8±0.5[a]	136.6[d]	52.08±2.80[b]
F-2	-	na	na
F-3	-	124.5[d]	52.21±2.30[c]
F-4	-	249.9[d]	51.55±3.40[c]
Cranberry			
R-E	10.6±0.4[a]	309.9[d]	45.37±2.52[b]
F-2	-	320.2[d]	41.29±9.56[c]
F-3	-	350.4[d]	60.40±6.33[c]
F-4	-	581.2/104.4[d]	43.94±2.60[c]
Cherry-Lapins			
R-E	5.4±0.4[a]	1422.7/174.1/19.4[d]	43.02±5.42[b]
F-2	-	105.8/18.1[d]	43.94±1.30[c]
F-3	-	189.8[d]	43.60±17.64[c]
F-4	-	1111.1/159.9[d]	57.38±4.00[c]
Cherry-Skeena			
R-E	11.0±0.3	1282.1/170.5/18.5	49.82±2.80
F-2	-	14.5	na
F-3	-	112.3	54.13±1.47
F-4	-	880/159	55.50±0.78
Cherry-Sweetheart			
R-E	4.7±0.3	999/117.2	35.20±5.11
F-2	-	93.5/17.7	na
F-3	-	118.3	57.71±2.00
F-4	-	800.7/141.5	58.83±4.52

R-E, raw polysaccharides; F-2, 0.05M NaCl eluate; F-3, 0.1M NaCl eluate; F-4, 0.25M NaCl eluate.
a, All values were determined according to the dry matter of extracted fruits.
b, All values were determined on the base of the dry weight of the respective vacuum dried raw polysaccharides.
c, All values were determined on the base of the dry weight of the respective lyophilized fraction.
d, All values were characterized on HPSEC containing Ultrahydrogel 120, 250 and 2000 (Waters, USA) in series in combination with a ultrahydrogel guard column and calculated according to dextran standards.
na, sample not available.
nd, not detectable.

Aqueous extracts of polysaccharides from the four fruits were further purified by ion-exchange chromatography on a 2.5×20 cm DEAE Sepharose Fast Flow column eluted with NaCl (0-0.5 M) gradient, and were separated into different fractions. The elution patterns of the different polysaccharides are presented in Fig. 1. The results showed that four fractions constituted the wolfberry polysaccharides, a finding consistent with earlier reports (Luo et al., 2004). Polysaccharides from the other three fruits studied displayed elution patterns very similar to that of wolfberry; however, wolfberry polysaccharides had a smaller fraction 2 and fraction 3, and kiwi polysaccharides also had a smaller fraction 2 peak compared to the cranberry and cherry polysaccharide fractions. Polysaccharides from different cherry

cultivars displayed the same elution pattern (data not shown). In fraction 1, polysaccharides were present only in trace amounts after dialysis and freeze-drying.

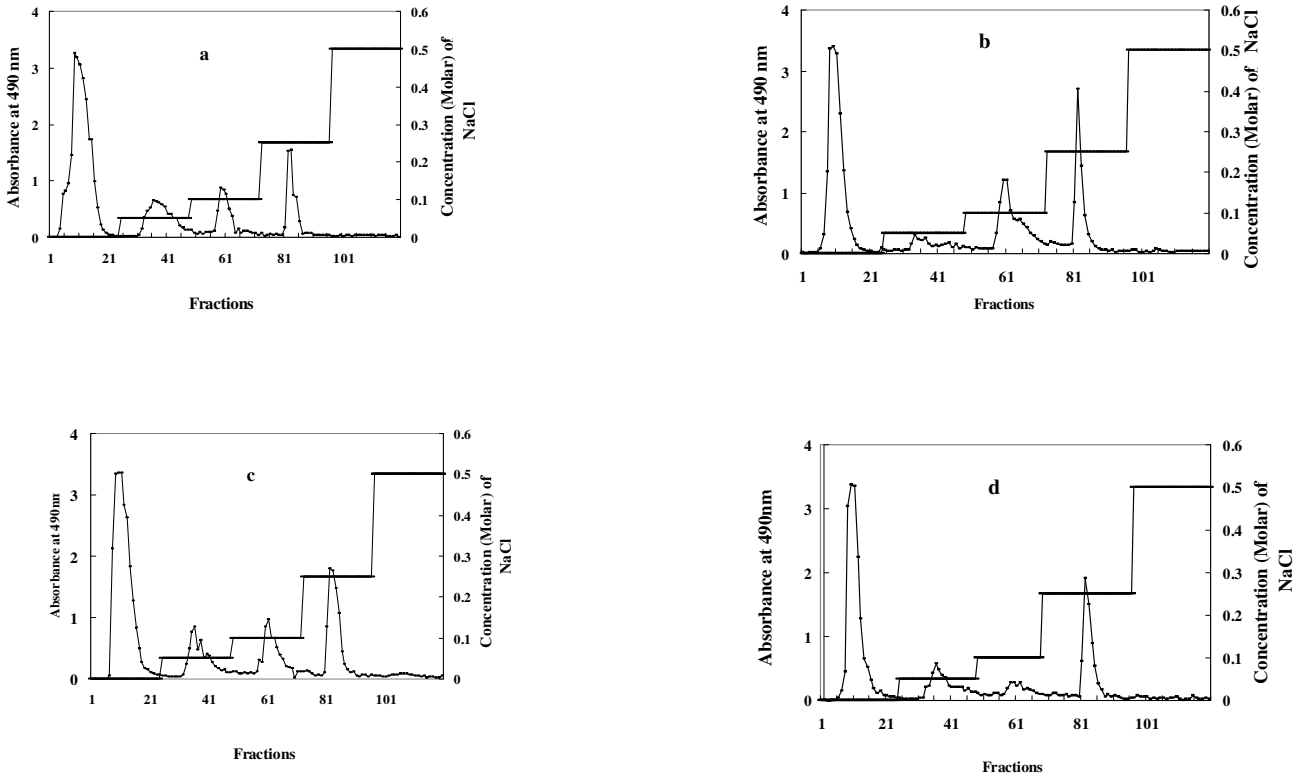

Fig.1. Elution patterns of polysaccharides from cranberry, kiwi, cherry and wolfberry on a DEAE Sepharose Fast Flow Column: a. Cranberry polysaccharides; b. Kiwi fruit polysaccharides; c. Lapins cherry polysaccharides; d. Wolfberry polysaccharides

Molecular weight distribution of polysaccharides

The molecular weight distribution of the polysaccharides in the raw extracts and their fractions are presented in Table 1. Two populations of polysaccharides were present in the raw extracts from wolfberry; their molecular weights were 604.5 kDa and 57.6 kDa, respectively. The raw extracts from "Lapins" cherries had three different populations of polysaccharides; their molecular weights were 1422.7 kDa, 174.1 kDa and 19.4 kDa, respectively. Raw extracts from "Skeena" cherries and "Sweetheart" cherries displayed molecular weight distribution patterns similar to "Lapins" cherries except that raw extracts of "Sweetheart" cherries did not show the low molecular population. This may reflect cultivar differences and/or differences in maturity. The raw extracts from kiwi and cranberries displayed a narrower molecular weight distribution range, with only one single symmetrical peak on their HPSEC chromatograms instead of one or two shoulder peaks as shown for wolfberry and cherry raw extracts (data not shown). The molecular weight of cranberry polysaccharides was 313 kDa, and the average molecular weight of kiwi fruit polysaccharides was 136.6 kDa. These indicate that the molecular distribution of the different polysaccharide fractions from kiwi and cranberry were very close to each other. Deters et al. (2005) also reported on the molecular composition of kiwi fruit; however, their molecular weight values are generally higher than our results. This can be attributed to differences in the extraction and separation procedures used. The results of HPSEC showed that the raw extracts from all three different cultivars of cherry and "Lapins" cherry harvested at three maturity levels had higher molecular weight polysaccharides than those of the other fruits investigated.

As expected, the molecular weights of the polysaccharide fractions were different for the different fractions (Table 1). Fraction 4 (F-4) from most of the fruits had more than one population of polysaccharides except for F-4 from kiwi fruit. The molecular weight of wolfberry fraction 2 (F-2) polysaccharides was about 56.6 kDa, a value very similar to that of reported by Peng and Tian (2001).

F-2 and fraction 3 (F-3) of wolfberry polysaccharides had a lower molecular weight than those of F-4. F-4 from wolfberry and cranberry polysaccharides also had portion of lower molecular weight near 100 kDa. F-4 from cherries had portion of lower molecular weight near 150 kDa, as well. The HPSEC results also showed that the molecular weight distribution of the fractions from the four different fruits displayed the same trend, even though they had different populations and also different distributions. Shoulder peaks or minor peaks on the HPSEC chromatograms of some fractions indicate that these fractions may still have sub-fractions.

Sugar composition of hot water extracted polysaccharides and their fractions

The sugar composition of hot water polysaccharides and their fractions obtained by DEAE Sepharose chromatography is summarized in Table 2. Sugar composition of fractions collected at different stages of the elution revealed that the composition of the eluted polysaccharides was different. This finding strongly suggests that the polysaccharides from the four fruits are heterogeneous.

Table 2. Sugar composition (mol %) of hot water extracted polysaccharides from wolfberry, kiwi, cranberry and cherry along with their fractions obtained by anion-exchange chromatography on DEAE Sepharose Fast Flow

Sample	Rhamnose	Fucose	Arabinose	Xylose	Mannose	Galactose	Glucose
Wolfberry							
R-E	0.47±0.07	0.10±0.03	8.84±0.76	0.57±0.05	0.22±0.01	1.65±0.13	1[a]
F-2	0.32[b]	0.45[b]	6.46[b]	0.83[b]	0.25[b]	1.57[b]	1
F-3	0.82[b]	0.52[b]	11.77[b]	0.83[b]	0.27[b]	3.29[b]	1
F-4	1.98±0.18	0.77±0.65	17.19±1.00	0.98±0.07	0.15±0.02	2.74±0.05	1
Kiwi							
R-E	0.40±0.03	0.26±0.02	7.14±0.02	0.71±0.06	0.3±0.03	2.64±0.24	1
F-2	na	na	na	na	na	na	na
F-3	0.93±0.14	nd	12.83±0.46	0.85±0.12	0.33±0.05	2.81±0.34	1
F-4	3.16±0.35	1.17±0.26	19.88±1.94	2.03±0.27	0.49±0.05	8.48±1.25	1
Cranberry							
R-E	0.16±0.01	t	4.38±0.42	1.5±0.26	t	2.01±0.40	1
F-2	0.37±0.17	t	3.17±0.11	0.71±0.22	0.08±0.02	1.26±0.42	1
F-3	0.57±0.06	nd	4.78±0.10	1.06±0.08	nd	2.77±0.18	1
F-4	2.99[b]	1.4[b]	25.62[b]	1.31[b]	0.2[b]	18.9[b]	1
Cherry-Lapins							
R-E	0.75±0.04	0.16±0.04	18.47±0.28	0.61±0.07	0.41±0.05	2.48±0.13	1
F-2	0.79±0.05	0.17±0.02	6.12±0.95	0.59±0.05	0.94±0.03	3.69±0.54	1
F-3	2.58±0.17	0.97±0.21	19.96±2.77	1.24±0.04	0.81±0.11	4.13±0.85	1
F-4	4.78±1.16	0.67±0.13	38.95±4.40	1.11±0.08	0.59±0.00	6.78±0.41	1
Cherry-Skeena							
R-E	1.12±0.21	0.24±0.05	10.85±1.22	0.49±0.05	0.59±0.01	2.89±0.45	1
F-2	0.54[b]	0.12[b]	1.50[b]	0.29[b]	0.76[b]	1.30[b]	1
F-3	0.67[b]	0.11[b]	3.45[b]	0.37[b]	0.76[b]	2.01[b]	1
F-4	1.69±0.17	0.29±0.01	28.69±7.47	0.87±0.17	0.81±0.06	6.47±0.94	1
Cherry-Sweetheart							
R-E	1.02±0.04	0.09±0.01	6.65±0.33	0.33±0.01	0.46±0.03	2.20±0.05	1
F-2	0.42[b]	0.08[b]	2.16[b]	0.45[b]	0.88[b]	2.19[b]	1
F-3	1.18±0.40	nd	7.90±0.10	0.68±0.07	0.85±0.06	5.35±0.34	1
F-4	3.62±0.06	0.24±0.01	32.11±2.55	0.87±0.03	0.40±0.07	7.28±0.45	1

R-E, raw polysaccharides; F-2, 0.05M NaCl eluate; F-3, 0.1M NaCl eluate; F-4, 0.25M NaCl eluate.
na , sample not available.
nd, not detectable.
t, trace
a, Mean values in a row for each sugar among the polysaccharides followed by the same letter were calculated relative to glucose in that polysaccharide sample.
b, Value was obtained from combined fractions of triplicate.

Rhamnose (Rha), arabinose (Ara), xylose (Xyl), galactose (Gal) and glucose (Glc) were the predominant component sugars in the hydrolysates of polysaccharides from wolfberry, cherry and kiwi, and fucose (Fuc) and mannose (Man) were present as minor sugar constituents. The relative molar ratio of wolfberry raw polysaccharides was 0.47: 0.10: 8.84: 0.57: 0.22: 1.65: 1.00 for Rha, Fuc, Ara, Xyl, Man, Gal and Glc, respectively. The predominant sugars of kiwi polysaccharides reported in published reports

are also Ara, Gal and Glc, although the molar ratios of the simple sugar composition from our study displayed some variance with respect to those of Deters et al. (2005). The sugar composition of hot water extracted polysaccharides from "Skeena", "Sweetheart" and "Lapins" cherries harvested at three maturity levels and their fractions also had different molar ratios. Cranberry raw polysaccharides were mainly composed of Rha, Ara, Xyl, Gal and Glc with relative molar ratios of 0.16: 4.38: 1.50: 2.01: 1. Neither Fuc nor Man was detected in the F-3 of cranberry polysaccharides, and no Fuc was detected in F-3 of the kiwi polysaccharides.

Antioxidant activity

Antioxidant activity of raw polysaccharides extracts and their fractions from the four different fruits evaluated by ORAC and TEAC assays are presented in Table 3. All raw polysaccharides extracts and their fractions (F-2, F-3 and F-4) had antioxidant activity.

Table 3. Antioxidant activity of different hot water extractable polysaccharides from wolfberry, kiwi, cranberry and cherry and their fractions

Sample name	ABTS (μmol Trolox equivalent/ g sample) [a]	ORAC (μmol Trolox equivalent/ g sample) [a]
Wolfberry		
Raw extracts	61.1±0.8	429.3±5.9
Fraction-4	23.3±3.0	99.9±8.8
Kiwi		
Raw extracts	129.0±6.7	320.6±9.1
Fraction-3	8.3±0.6	67.4±7.1
Fraction-4	6.1±0.3	67.9±3.8
Cranberry		
Raw extracts	137.4±3.7	310.9±36.0
Fraction-2	7.8±1.0	12.8±1.1
Fraction-3	3.2±0.2	38.7±7.1
Fraction-4	2.8[b]	91.9±8.3
Lapins cherries		
Raw extracts	159.3±17.8	535.8±6.4
Fraction-2	3.2[b]	34.0±4.7
Fraction-3	3.5±1.2	21.1±1.6
Fraction-4	7.2±1.0	79.4±15.6

a, All values were determined on the basis of the dry weight of the respective vacuum dried raw polysaccharides.
b, The value was obtained from a combined fraction because of the sample availability.

The antioxidant activity of the raw polysaccharide extracts and their fractions varied with the type of fruit and with the fraction. Antioxidant activity determined by the ORAC method showed higher values than that of the TEAC assay, but this only reflected the difference in the sensitivity of the two methods. The data showed that all raw polysaccharides from the four types of fruit had higher antioxidant activity than their fractions. This is likely due to the fact that the raw extracts contained other components in addition to the polysaccharides such as polyphenols, proteins, amino acids, peptides, carotenoids, riboflavin and/or ascorbic acid which were removed by DEAE Sepharose chromatography. However, the finding that the purified polysaccharide fractions, F-2, F-3 and F-4 from cherries and cranberries, F-3 and F-4 from kiwi, and F-4 from wolfberries, had antioxidant activity is most interesting, and indicates the potential beneficial effect of fruit polysaccharides as antioxidants.

Raw polysaccharide extracts from sweet cherries showed the highest antioxidant activity of the four fruits investigated. Antioxidant activity results of raw polysaccharides from "Lapins" cherries harvested at different maturity levels also showed that antioxidant activity of cherry water-soluble polysaccharides increased during ripening. Wolfberry raw polysaccharides extracts had higher antioxidant activity than that of kiwi and cranberry fruits but lower than cherries. Also, the ORAC values showed that F-3 and F-4 of the kiwi polysaccharides had similar antioxidant activity; however, F-4 of cranberry and also cherry polysaccharides had higher ORAC values compared to the F-2 and F-3 of the respective polysaccharides. These results suggested that the molecular weights of the polysaccharides may play an important role on their antioxidant activity. A relatively higher molecular weight of the cherry and cranberry F-4 fractions may have resulted in an increase in the antioxidant activity of these fractions.

To the best of our knowledge, there are no published

reports on the antioxidant activity of cherry, kiwi and cranberry polysaccharides, and thus no comparison between the results of this study and literature values can be made. However, our antioxidant activity results for wolfberry polysaccharides are in full agreement with results reported by Luo et al. (2004) who found that wolfberry fruits extracts/fractions possess antioxidant activity, and can significantly reduce blood glucose levels and serum total cholesterol and triglyceride concentrations after 10 days treatment in rabbits, indicating that there are substantial hypoglycemic and hypolipidemic effects. Similarly, Li et al. (2007) evaluated the antioxidant activity of wolfberry polysaccharides by several procedures and concluded that these polysaccharides have multiple antioxidant activity as illustrated by their significant reducing power, superoxide scavenging ability, inhibition of mice erythrocyte hemolysis mediated by peroxyl free radicals and also ferrous ion chelating potency.

There are very few reports in the literature on the structure/activity relationship of the polysaccharides under analysis. However, a very recent publication by Ajisaka et al. (2009) on antioxidant potency of various carbohydrate molecules, including mono-, di-, oligo-, and polysaccharides of various structures, concluded that with the exception of agaro-oligosaccharide, all of the tested carbohydrates that showed antioxidant activity possessed either an amino, carboxyl, carbonyl, or sulfonyl group.

The antioxidant activity results obtained in our study clearly established the antioxidant potency of the polysaccharides extracted from wolfberry, cherry, kiwi and cranberry fruits. However, the relationship between the structure of fruit polysaccharides and antioxidative mechanisms requires further studies.

Conclusions

HPSEC chromatography of polysaccharides from sweet cherry, wolfberry, kiwi and cranberry fruits showed that all four fruits' polysaccharides were separated into four fractions on DEAE Sepharose chromatograph. Polysaccharides from cherries had higher molecular weights than those from other fruits. Different fruit polysaccharides had different ratios of simple sugars although they were all mainly composed of Rha, Ara, Xyl, Gal and Glc. TEAC and ORAC antioxidant assays showed raw polysaccharides from cherries exhibited the highest antioxidant activity among the four fruits' raw polysaccharides. The results suggest that polysaccharides from the four fruits studied are very similar and display antioxidant activity.

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