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Cell wall polysaccharides of Chinese Wolfberry (*Lycium barbarum*): Part 1. Characterisation of soluble and insoluble polymer fractions

Robert J. Redgwell[a,*], Delphine Curti[a], Juankuan Wang[a], Justyna M. Dobruchowska[b], Gerrit J. Gerwig[b], Johannis P. Kamerling[b], Peter Bucheli[c]

[a] *Nestlé Research Centre, Vers-Chez-Les-Blanc, 1000 Lausanne 26, Switzerland*
[b] *Department of Microbiology, University of Groningen, Kerklaan 30, NL-9751 NN Haren, Netherlands*
[c] *Nestlé Product Technology Centre, Nestlé-Strasse-3, 3510 Konolfingen, Switzerland*

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ABSTRACT

Water-soluble polysaccharides (WSP) and insoluble cell wall material (CWM) were isolated from Wolfberry fruit (*Lycium barbarum*). WSP were fractionated by treatment with a quaternary ammonium salt and anion-exchange chromatography. Pectic polysaccharides were major components but a glucan, xylan and arabinogalactan-proteins (AGP) were also present. CWM was fractionated into pectic and hemicellulosic polysaccharides by a sequential solvent extraction. The former were rhamnogalacturonans with varying degrees of branching of the backbone. A (4-*O*-methyl-glucurono)xylan was the predominant hemicellulose. The additional presence of xyloglucan and a mannan-based polymer, strongly attached to the cellulose fibrils was indicated. Of the 6.8% total polysaccharide content of Wolfberry fruit, 1.2% was readily water soluble, 5.6% insoluble and 0.3% was readily soluble AGP.

1. Introduction

Wolfberry is a dicotyledon belonging to the *Solanaceae* family which includes such crops as potato, tomato, peppers and tobacco. There are two common species: *Lycium barbarum* and *Lycium chinense* which produce an orange-red ellipsoid berry 1–2 cm long. Although the Wolfberry plant is grown in many different regions of the world it is commercially produced only in China which remains the major supplier of Wolfberry products worldwide. In the West, recent marketing of Wolfberry products (or goji berry as it is often called) has highlighted the reputation of Wolfberry polysaccharides as having extensive biological effects and potential health benefits.

Wolfberries have been used in Chinese medicine for over 2000 years where their beneficial effects are claimed to enhance the immune system, improve eye sight, and circulation, and to possess anticarcinogenic properties. The immune enhancing, anti-cancer and antioxidative properties of Wolfberry polysaccharides are reported in several Chinese publications (Gan, Zhang, Yang, & Xu, 2004; Gau, Yang, & Du, 1994; Geng, Xing, Zhou, & Chu, 1989; Huang, Lin, Tian, & Ji, 1998; Li & Zhou, 2007) and have been attributed to a group of glycoconjugates identified as arabinogalactan-proteins (AGP).

Huang, Tian, and Zheng (1999) reported the major structural features of a Wolfberry AGP as a (1 → 4)-β-D-galactan substituted at O-3 with α-L-arabinosyl side chains. A second glycoconjugate (LbGp4) confirmed this basic structure (Peng, Huang, Qi, Zhang, & Tian, 2001). However, a later publication (Zhang & Zhang, 2007) reported that an AGP (LBP-4) contained high amounts of galacturonic acid, suggesting that LBP-4 may contain more than one type of polysaccharide. Furthermore, it was reported the galactan backbone contained (1 → 3) linkages. The most recent evidence for the presence of pectic polysaccharides as a major component of Wolfberry polysaccharides was provided by Zou, Zhang, Yao, Niu, and Gao (2010), who reported on the hypoglycemic activity of a polysaccharide fraction composed of rhamnose, arabinose, xylose, galactose, mannose and galacturonic acid. This would suggest that the fraction contains more than one type of polysaccharide. To date nothing has been reported on the water-insoluble polysaccharides of Wolfberry.

In the present study the total polysaccharide (soluble and insoluble) content was isolated, fractionated and subjected to compositional and linkage analysis to identify the major polysaccharide types in Wolfberry fruit.

* Corresponding author. Tel.: +41 21 785 8681.
E-mail address: robert.redgwell@rdls.nestle.com (R.J. Redgwell).

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2. Materials and methods

2.1. Plant material

Wolfberries (*L. barbarum*) were obtained as dried fruit (water content ~3.0%) from Ningxia Chinese Wolfberry Group Company, Ningxia province, China.

2.2. Reagents

The β-D-glucosyl Yariv reagent [1,3,5-tris (4-β-D-glucopyranosyloxyphenylazo)-2,4,6-trihydroxy-benzene] was purchased from Biosupplies Australia Pty Ltd.

2.3. General methods

Monosaccharide composition of polysaccharide fractions was determined by capillary GLC of the alditol acetate derivatives following acid hydrolysis in either 72% sulfuric acid (3 h at room temperature) and then 1 M sulfuric acid (2 h at 110 °C), or 2 M trifluoroacetic acid (TFA, 1 h at 110 °C). Uronic acid was determined quantitatively by colorimetric assay (Blumenkrantz & Asboe-Hansen, 1973). Since this procedure does not discriminate between glucuronic and galacturonic acids, polysaccharide hydrolysates made in sulfuric acid were analysed by HPAEC-PAD on a Dionex SLC 3000 instrument according to the SOP 4149-1 procedure using a CarboPac PA-1 column (4 mm × 250 mm) equilibrated with 150 mM NaOH.

Methylation analysis used a modification of the method of Ciucanu and Kerek (1984). Carboxyl groups of the uronic acid residues were reduced to the corresponding 6,6-dideuterioglycosyl residues (Kim & Carpita, 1992). GLC–MS of the partially methylated alditol acetates was accomplished as described previously (Redgwell, Melton, & Brasch, 1988).

2.4. Yariv plate assay

The Yariv plate assay (Van Holst & Clarke, 1985) was used to monitor the presence of AGP. A solution of 0.001% Yariv reagent, 0.02% sodium azide, 0.15 M sodium chloride and 1% agarose was heated to dissolve the agarose and stored in the refrigerator pending use. For an assay the Yariv agarose gel was melted by heating and poured into a Petri dish to give a 3 mm thick layer of gel. Wells (3–4 mm wide) were made in the gel and 15 μL of test solution (4 mg/mL in 0.15 M NaCl) introduced into each well. A solution of arabic gum was included as a positive control. The Petri dishes were sealed with a Parafilm and stored in the darkened cupboard for 2 days to allow the red halos to develop.

2.5. Isolation of Wolfberry polysaccharides

Isolation and fractionation of the carbohydrate content of Wolfberry was done as set out in Fig. 1. Approximately 20 g of Wolfberries were cryo-milled in liquid nitrogen. The powder was transferred to a flask with 100 mL of 80% EtOH and refluxed for 5 min. The cooled suspension was transferred into a dialysis membrane (3.5 kDa molecular weight cut-off membrane) and dialysed for 12 h with changes of H_2O (1 L each time). The 3 L of dialysate H_2O were combined, concentrated and freeze dried to give the low molecular weight fraction. The dialysis was carried on for a further 3 days with regular changes of water every 24 h (20 L each time). The contents of the dialysis bag were separated by centrifugation (7000 rpm, 15 min) into a supernatant and an insoluble residue. The latter was washed once in distilled water and following centrifugation the supernatant added to the first supernatant. The combined supernatants were concentrated and freeze dried to give a water-soluble polysaccharide fraction (WSP). The insoluble residue was freeze dried to give the insoluble cell wall material (CWM).

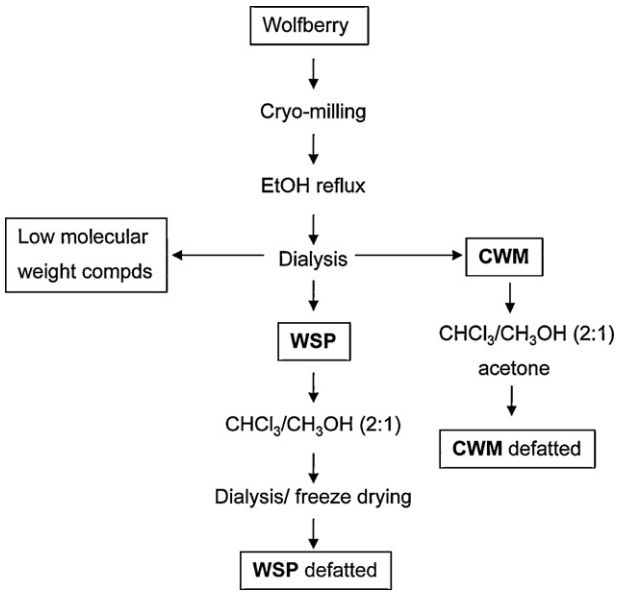

Fig. 1. Scheme for isolation of WSP and CWM of Wolfberry.

2.6. Defatting of WSP

Freeze dried WSP (3.56 g) was suspended in chloroform/methanol (2:1 v/v, 600 mL) and stirred for 30 min at ambient temperature. The fat-soluble material in the supernatant was separated by centrifugation (10 min at 7000 rpm) and the extraction repeated. The insoluble residue was then resuspended in acetone, stirred for 30 min and the supernatant discarded after centrifugation. The de-fatted polysaccharide fraction remaining after the extraction was dissolved in a little water, dialysed overnight (3.5 kDa molecular weight cut-off membrane) and recovered after freeze drying. The CWM was defatted in an identical manner.

2.7. Fractionation of CWM

Defatted CWM was fractionated by sequential extraction with 0.05 M trans-1,2-diaminocyclohexane-*N*,*N*,*N*′,*N*′-tetraacetic acid (CDTA; 6 h), 0.05 M Na_2CO_3 (18 h, 4 °C and 2 h, ambient temperature) and 4 M KOH (2 h, ambient temperature) (Redgwell et al., 1988). All alkaline solvents contained 20 mM sodium borohydride. Solutions (and the final insoluble residue) were recovered following neutralisation to ~pH 5.0 with acetic acid, dialysis and freeze drying.

2.8. Treatment of WSP with quaternary ammonium salt

The quaternary ammonium salt, hexadecyltrimethylammonium bromide (CTAB), is able to precipitate acidic polysaccharides from aqueous solution providing they contain a moderate amount of uronic acid (Scott, 1965). AGP which have a uronic acid content of ~10% or less are not precipitated. Thus, the method provides a convenient way of separating AGP from the more acidic pectic polysaccharides present in the WSP.

Defatted WSP was dissolved in 200 mL of water and 200 mL of 1% CTAB added with stirring. The precipitate was allowed to settle overnight at 4 °C and recovered by centrifugation (7000 rpm,

Table 1
Monosaccharide composition of WSP and CWM fractions.

Fraction	Monosaccharide composition (mole%)								Total (μg/mg)
	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	HexA	
WSP	1.6	0.2	28.0	3.1	0.8	13.8	9.5	43.2	500.0
CWM	0.7	0.1	9.2	24.2	8.6	3.9	31.2	22.2	367.0

15 min). The supernatant, which contains the non-pectic fraction, was dialysed in a 3.5 kDa membrane against 5 L of 50% ethanol for 24 h (ethanol changed 3 times) and then dialysed for a further 2 days against water. The polysaccharide was recovered by freeze drying to give WSP1.

The precipitate was dispersed in 20 mL of 4 M NaCl and 300 mL of ethanol saturated with sodium acetate added. The precipitated material was allowed to settle and the supernatant discarded. The step was repeated several times over 24 h and finally the polysaccharide pellet was dissolved in water, dialysed for three days in a 3.5 kDa membrane against water at 4 °C and freeze dried to give WSP2.

2.9. Anion-exchange chromatography

Chromatography of soluble polysaccharide fractions was done on columns of DEAE-Sepharose (2.5 cm × 15 cm) equilibrated in 0.05 M NH_4HCO_3. The sample (200–300 mg) was dissolved in 50 mL of 0.05 M NH_4HCO_3, eluted on to the column bed and followed sequentially by 250 mL of 0.05, 0.1, 0.2, 0.4 and 0.8 M NH_4HCO_3. Each fraction was concentrated on the rotary evaporator before freeze drying. After the first freeze drying the samples were weighed and then redissolved in a little water and freeze dried again. This ensured complete removal of the NH_4HCO_3.

3. Results and discussion

3.1. Isolation and composition of WSP and CWM

Water-soluble polysaccharides (WSP) and the insoluble residue (CWM) accounted for ~3% and 17%, respectively, of the original berries. The low molecular weight compounds which passed through the dialysis membrane accounted for 60% of the original berries.

Approximately 70% of the low molecular weight fraction consisted of glucose, fructose and sucrose in the ratio of 8:8:1. The WSP consisted predominantly of uronic acid, arabinose and galactose (Table 1), indicative of pectic polysaccharides, arabinans and/or arabinogalactans. As expected the carbohydrate part of the CWM was made up of mainly glucose (derived principally from cellulose). The major non-cellulose derived sugar was xylose, indicating the presence of a xylan. This fact, together with the presence of mannose, pointed to moderate amounts of hemicellulosic polysaccharides in the CWM.

The polysaccharide content of the WSP and CWM accounted for 50% and 36.7% of the total fractions. An additional 17.0 and 25% of the WSP and CWM, respectively, was protein. Unaccounted for material was not identified but polyphenolics and low molecular weight compounds which absorbed to the fractions during dialysis could be contributory elements.

3.2. Fractionation of WSP

The monosaccharide composition of the WSP indicated the presence of a mixture of acidic and neutral polysaccharides. It was therefore subjected to treatment with CTAB to separate the polysaccharides into acidic and neutral fractions. CTAB precipitated the pectic polysaccharides from WSP (WSP2) leaving a supernatant fraction enriched in AGP (WSP1). The Yariv plate assay demonstrated that only trace amounts of AGP co-precipitated with the pectic fraction (Fig. 2). WSP1 contained polysaccharides which consisted of mainly arabinose and galactose in a ratio of 2:1 (Table 2). This fraction also contained most of the glucose, xylose and mannose found in the WSP. WSP2 contained predominantly uronic acid, indicating pectic polysaccharides.

Both WSP1 and WSP2 were submitted to anion-exchange chromatography on DEAE-Sepharose. Fractions were recovered by sequential elution with increasing concentrations of NH_4HCO_3 solution and their monosaccharide composition determined (Table 2). All fractions of WSP1 were rich in arabinogalactans as arabinose and galactose accounted for >65% of the monosaccharides. However, the relative amount of each varied considerably. The 0.05 M fraction had an arabinose:galactose ratio of ~3:1 whereas for the 0.1 M fraction the ratio was ~1:1 and for the 0.4 M fraction ~2:1. All fractions gave a strong positive result for AGP when tested in the Yariv plate assay (Van Holst & Clarke, 1985). However, the moderate levels of xylose and glucose in some fractions (sugars not normally found in AGP) were an indication that other polysaccharides were present. In WSP2, 20% of polysaccharides were not retained on the column despite the high level of uronic acid (Table 2). This behaviour has previously been ascribed to aggregation of the pectin molecules via interchain hydrogen bonding of the non-esterified carboxyl groups (Redgwell & Selvendran, 1986). In those fractions which did bind to the column the uronic acid was the predominant component and its content varied between 50 and ~90% of the molecule. The rhamnose/uronic acid ratio also differed significantly among the fractions. These results are consistent with the idea that Wolfberry contains a mixture of pectic polysaccharides, the predominant structural feature of which is a

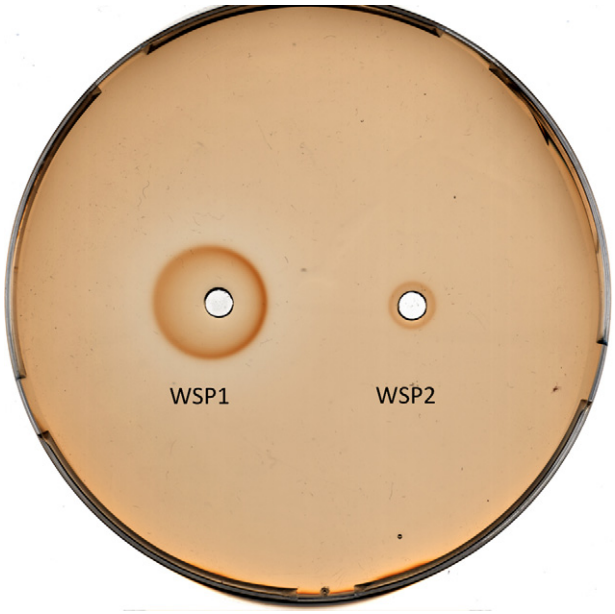

Fig. 2. β-D-Glucosyl Yariv plate assay for AGP in CTAB-supernatant (WSP1) and CTAB-precipitate (WSP2).

Table 2
Monosaccharide composition of WSP1 and WSP2 and subsequent fractions obtained from anion exchange chromatography of each on DEAE-Sepharose.

Fraction	% yield	Monosaccharide composition (mole%)								Total (μg/mg)
		Rha	Fuc	Ara	Xyl	Man	Gal	Glc	HexA	
WSP1	100	1.6	0.2	51.4	4.8	1.2	25.9	7.3	7.4	514.2
0.05 M	39.5	0.4	0.2	60.0	5.1	2.1	18.2	11.0	2.8	464
0.1 M	15.8	2.3	0.1	38.5	8.7	0.5	34.7	5.3	10.0	545
0.2 M	23.5	3.4	0.2	42.9	2.1	0.4	33.2	3.8	14.1	547
0.4 M	10.7	3.7	0.3	44.3	1.1	0.6	22.9	9.8	17.4	325
0.8 M	3.9	1.5	0.3	50.7	3.8	1.8	16.6	19.3	6.0	236
WSP2	100	1.4	0.1	12.3	1.8	0.2	6.3	1.5	76.0	663.2
0.05 M	20.6	1.1	0.1	15.3	3.2	0.3	5.9	1.5	72.6	730
0.1 M	2.0	1.5	0.1	14.5	11.7	0.9	11.3	9.6	50.5	529
0.2 M	24.3	2.2	–	12.1	0.7	–	10.1	1.3	73.5	572
0.4 M	27.6	1.1	0.1	5.0	0.6	–	2.5	0.6	90.1	915
0.8 M	7.1	0.6	0.1	7.8	2.0	–	1.2	1.5	86.8	734

rhamnogalacturonan backbone, with variable degrees of substitution with galactosyl and arabinosyl residues.

3.3. Linkage analysis

Analysis of the partially methylated alditol acetates in WSP, WSP1 and WSP2 was done on carboxyl-reduced polysaccharides to allow assignments to be made for the uronic acid components (Table 3). Since no 4-substituted galactopyranosyl residues were detected in the unreduced WSP, 4-substituted galactopyranosyl residues detected in the carboxyl-reduced WSP must have been derived from the reduced carboxyl group of galactopyranosyluronic acid residues. The fact that they accounted for more than 40% of the linkage types indicates that pectic polysaccharides accounted for a major part of the WSP in Wolfberry. Following enrichment of the acidic polysaccharides in WSP2 by CTAB precipitation the 4-substituted galacturonic acid content increased to 68%.

The structural features of type II arabinogalactans found in many AGP consist for the most part of a backbone of (1 → 3)-linked β-D-galactopyranosyl residues, some of which are substituted at O-6 with α-L-arabinofuranosyl and β-D-galactopyranosyl residues (Fincher, Stone, & Clarke, 1983). The linkage analysis of WSP1 accords with these reports and is consistent with the idea that Wolfberry AGP is made up of a backbone of (1 → 3)-linked galactopyranosyl residues, substituted at intervals in the O-6 position with various combinations of mostly arabinofuranosyl and galactopyranosyl residues. The ratio of 3-substituted to 3,6-disubstituted galactopyranosyl residues (1:2.6) indicated a highly branched molecule. The side chains existed predominantly as 5-substituted arabinofuranosyl residues but the complex nature of the side chains is indicated by the presence of 2-substituted, 3-substituted, and 3,5-disubstituted arabinofuranosyl residues. Although arabinofuranosyl residues appear to terminate most of the side chains, galactopyranosyl, rhamnopyranosyl and glucopyranosyluronic acid residues also occur as terminal sugars. In addition to terminal glucopyranosyluronic acid residues, some 4-substituted glucopyranosyluronic acid residues were identified. Whether these are structural features of Wolfberry AGP or components of a separate polysaccharide will require additional purification of the AGP. The additional presence of 4-substituted xylopyranosyl and glucopyranosyl residues in WSP1 indicated the presence of additional types of polysaccharide.

Table 3
Linkage analysis of WSP, and WSP1 and WSP2.

Sugar	Linkage	WSP (mole%)	WSP1	WSP2
Rha*p*	Terminal	1.2	1.8	1.0
Ara*f*	Terminal	11.8	19.8	5.0
	2-	5.0	13.2	2.2
	3-	2.6	4.7	0.4
	5-	7.1	12.8	9.5
	3,5-	0.6		
Xyl*p*	Terminal		1.0	
Xyl*p*	4-	2.8	3.0	
Gal*p*	Terminal	1.0	1.7	1.4
	3-	3.7	7.3	1.5
	6-	1.0	2.8	
	3,6-	8.9	20.5	4.8
Glc*p*	Terminal	1.7	0.7	0.6
	4-	5.2	5.4	2.6
	4,6-	1.7	1.8	
Gal*p*A[a]	4-	43.3	1.3	68.0
	3,4-	1.8		2.6
Glc*p*A[a]	Terminal	0.3	1.6	0.4
	4-	0.7	3.9	0.7

[a] Glucuronic and galacturonic acids analysed as 6,6-dideuterio-glycosyl residues following carboxyl reduction of the polysaccharides with sodium borodeuteride.

3.4. Fractionation of CWM

CWM was fractionated by sequential extraction with CDTA (a chelating agent), 0.05 M Na_2CO_3 and 4 M KOH. CDTA is aimed at solubilising the homogalacturonans of the middle lamella while Na_2CO_3 releases some of the primary cell wall pectic polysaccharides. Strong alkali can solubilise hemicelluloses or polysaccharides which are strongly hydrogen bonded to the cellulose fibrils.

3.4.1. Pectic polysaccharides

The amounts of the soluble fractions recovered from each extractant and their monosaccharide compositions are given in Table 4. The polysaccharide in the CDTA-soluble fraction contained 76.3% uronic acid which is consistent with it being a homogalacturonan type polysaccharide commonly found in the middle lamella of dicotyledonous plants. The Na_2CO_3-soluble fraction was also rich in uronic acid but contained a higher proportion of neutral sugar residues which is to be expected in the more highly branched pectic polysaccharides found in the primary cell wall. This is supported by the increased amounts of rhamnose which commonly occur as branch points in the rhamnogalacturonan backbone of pectic polysaccharides.

3.4.2. Hemicellulosic polysaccharides

The 4 M KOH-soluble fraction was rich in xylose and glucose. This is not unexpected as xyloglucans, arabinoglucuronoxylans and galactoglucomannans are ubiquitous hemicelluloses in the primary cell wall of dicotyledonous plants (Redgwell & Fischer, 2002). However, the xylose content of the fraction is twice that of the glucose

Table 4
Yield and monosaccharide composition of CWM fractions.

Fraction	% CWM[a]	Monosaccharide composition (mole%)								Total (μg/mg)
		Rha	Fuc	Ara	Xyl	Man	Gal	Glc	HexA	
CDTA-sol	8.4	1.2	0.2	15.9	1.0	0.5	4.0	1.0	76.3	545
Na_2CO_3-sol	5.5	3.3	0.3	33.7	2.0	0.7	6.6	1.5	51.9	331
4M KOH-sol	11.2	1.8	0.2	19.1	31.9	4.8	10.1	15.1	18.0	346
Residue-sol[b]	2.9	4.7	0.3	41.1	3.9	–	12.4	2.4	35.1	347
Residue	53.1	0.6	–	5.8	28.9	11.1	2.7	44.5	6.5	396

[a] The remaining 18.7% of the CWM was recovered as insoluble material which precipitated in each fraction during dialysis.
[b] During dialysis of the final residue additional material was solubilised. It was recovered as a separate fraction from the insoluble residue.

Table 5
Linkage analysis of 4 M KOH-soluble fractions from CWM.

Sugar	Linkage	4 M KOH	4 M KOH CTAB-ppt	4 M KOH CTAB-soluble
Rha*p*	3,4-	1.0	1.4	–
Ara*f*	Terminal	6.0	3.7	8.4
	2-	2.7	–	2.7
	3-	0.7	–	–
	5-	7.8	3.3	4.0
	3,5-	0.1	2.7	–
Xyl*p*	Terminal	3.0	1.7	6.0
	4-	43.8	75.0	10.7
	2,4-		4.8	–
Gal*p*	Terminal	6.0	1.3	10.4
	3-	1.6	–	2.0
	4-	1.0	1.0	–
	3,6-	2.0	0.9	2.0
Glc*p*	4-	14.4	2.6	25.5
	4,6-	7.0	0.3	13.7
Man*p*	4-	2.2	–	6.0
	4,6-	3.0	–	5.6

which suggests that a xylan and not xyloglucan is the predominant polysaccharide in the 4 M KOH fraction. The fraction was subjected to CTAB precipitation which separated the polysaccharides into acidic (precipitated fraction) and neutral (supernatant) fractions. These and the original 4 M KOH fraction were subjected to linkage analysis (Table 5).

The neutral fraction of the 4 M KOH fraction contained 4-substituted and 4,6-disubstituted glucopyranosyl and mannopyranosyl residues and moderate amounts of terminal galactopyranosyl residues. These residues can serve as the basic building blocks of xyloglucans and/or galactoglucomannans.

The acidic fraction is dominated by the presence of 4-substituted xylopyranosyl residues. The acidic nature of this fraction was confirmed by anion-exchange chromatography on DEAE-Sepharose (Table 6). More than 70% of the fraction bound to the column and was recovered only after elution with concentrated solutions of NH_4HCO_3. To identify the uronic acid in the 0.1, 0.2 and 0.4 M fractions, samples were subjected to reduction of the carboxyl groups, converting the uronic acid to the corresponding neutral sugar. Owing to the small amount of material the 0.1 M and 0.2 M fractions were combined. Following reduction the mixture of 0.1 M and 0.2 M fractions were subjected to acid hydrolysis and showed a marked increase in the proportion of glucose and the appearance of 4-*O*-methyl-glucose. This result together with the occurrence of 4-substituted and 2,4-disubstituted xylopyranosyl residues was evidence for the existence of a (4-*O*-methyl-glucurono)xylan in Wolfberry cell walls. Approximately ~40% of the glucuronic acid occurred as the endogenously methylated derivative.

In contrast, the reduced 0.4 M fraction showed only an increase in the proportion of galactose indicating that the uronic acid originated solely from galacturonic acid and that the majority of the polysaccharides in the 0.4 M fraction were pectic in origin.

The residue-soluble fraction had quite a different composition to that of the 4 M KOH fraction, the major components being arabinose and uronic acid and an elevated level of rhamnose. This is consistent with a highly branched pectic polysaccharide which past work has shown is extremely difficult to extract or solubilise from the cell wall (Redgwell, Fischer, Kendal, & MacRae, 1997). The 4 M KOH treatment causes the cellulose fibrils to swell and this allows the slow solubilisation of these pectic polysaccharides during the subsequent dialysis step.

The final residue consisted mostly of cellulose-derived glucose but moderate amounts of xylose and mannose indicated the presence of hemicelluloses.

The Yariv plate assay showed that some AGP existed in all the CWM fractions. There are two likely causes for the failure of a prolonged water extraction to solubilise 100% of the Wolfberry AGP. Some AGP could be covalently linked to the pectic polysaccharides or alternatively are entrapped in the fibrillar structure of the cell wall matrix. To test the first possibility some of the CDTA-, Na_2CO_3- and 4 M KOH-soluble fractions were subjected to CTAB treatment and the precipitated pectic polysaccharides and the non-precipitated fraction subjected to the Yariv plate assay. Only a trace of AGP was detected in the pectic polysaccharide fraction, evidence that the majority of AGP were not covalently linked to the pectic polysaccharides. It seems therefore that some AGP are trapped within the network structure of the cell wall and are released when part of this structure breaks down or during a more severe solvent extraction. A precedent for this phenomenon has been documented for the AGP in coffee bean cell walls where most of the AGP is only released following enzyme treatment to break down the cellulose fibrils (Redgwell, Curti, Fischer, Nicolas, & Fay, 2002).

Table 6
Fractionation on DEAE-Sepharose of CTAB-precipitated material from 4 M KOH-soluble fraction.

Fraction	Monosaccharide composition (mole%)								Total (μg/mg)
Yield (mg)	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	HexA	
Original	3.0	0.1	17.5	43.9	0.3	5.3	2.6	27.4	674
0.05 M 27.1	3.9	0.1	26.0	28.2	0.6	6.4	4.3	30.4	834
0.1 M 7.9	0.4	0.04	3.4	74.1	0.8	1.1	1.2	18.8	646
0.2 M 11.3	1.3	0.06	13.8	55.6	1.7	6.3	1.8	19.4	621
0.4 M 29.9	4.0	0.2	26.0	25.1	1.3	10.9	3.3	29.3	598
0.8 M 39.7	5.4	0.2	22.5	9.2	0.7	6.0	3.0	53.0	604

4. Conclusions

Based on the weight of recovered fractions and their polysaccharide content determined from monosaccharide analysis of acid hydrolysates the following observations can be made on the nature and amount (wt%) of the polysaccharide content of dried Wolfberries (3.3% water):

- Total polysaccharide 6.8%
- Readily water-soluble polysaccharide 1.2%
- Insoluble polysaccharide 5.6%
- Readily water-soluble AGP 0.3%

The major polysaccharides in WSP were pectic polysaccharides. This was predictable as solubilisation of pectic polysaccharides of the cell wall is a widespread phenomenon during fruit ripening for many species (Redgwell & Fischer, 2002). AGP accounted for only 20% of WSP. At least three additional types of polysaccharide were present. These included a xylan, a glucan and an arabinan.

The insoluble CWM of Wolfberry contained pectic polysaccharides with various degrees of branching of the rhamnogalacturon backbone. However, the major polysaccharides (apart from cellulose) in the CWM were xylose-based polysaccharides. In dicotyledenous fruit it is common to find xyloglucan as the major hemicellulose of the primary cell wall. This polysaccharide may also be present in Wolfberry but the major hemicellulose was a xylan and evidence indicated it to be a (4-*O*-methyl-glucurono)xylan. Moderate levels of mannose in the CWM pointed to the existence of a mannan-based hemicellulose. Most of this was strongly attached to the cellulose fibrils as evidenced by its failure to solubilise even in 4 M KOH.

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