

The Carbohydrate Composition of Cotyledons and Hulls of Cultivars of *Lupinus angustifolius* from Western Australia

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(Received 1 July 1992; accepted 30 October 1992)

Abstract: The hulls and cotyledons from three Western Australian cultivars (Gungurru, Yorrel and Danja) of *Lupinus angustifolius*, all of low alkaloid content, were analysed separately for their carbohydrate content and composition. Only minor differences in composition between these three cultivars were observed. More notably, the cotyledons of all the cultivars contained levels of non-starch polysaccharides (NSP), ranging from 290 to 310 g kg⁻¹ dry weight considerably higher than had been measured previously in cultivars of this species. Galactose, arabinose and uronic acid residues accounted for approximately 67%, 13% and 10%, respectively, of the cotyledon NSP. Although only a small proportion of the cotyledon NSP is soluble, a much larger proportion could be extracted with hot EDTA treatment. The oligosaccharide content of the cotyledons ranged from 74 to 80 g kg⁻¹ dry weight. Cotyledons had very low contents of cellulose, lignin and starch. Hulls consisted predominantly of NSP, with values ranging from 856 to 891 g kg⁻¹ dry weight. Glucose, xylose, uronic acids and arabinose were the principal sugar residues present reflecting the compositions of the major constituent polysaccharides, cellulose, hemicelluloses and pectins. Only low levels of lignin were measured in hulls. Cotyledon NSP and hulls from these cultivars may have considerable value as sources of dietary fibre in the human diet.

Key words: lupin, cotyledon, hull, carbohydrate, composition, polysaccharide, oligosaccharide, dietary fibre.

INTRODUCTION

Plant breeding programmes in Western Australia have successfully produced cultivars of the narrow-leaved lupin, *Lupinus angustifolius* L, of very low alkaloid content which are well adapted to local soils and climate (Ayling and Johnston 1988). Production of these cultivars has now increased to the extent that Australia has become the leading international exporter of lupin grain. While most of this commodity is sold into the livestock feed market, since 1990 (NHMRC 1991), lupins have gained acceptance in Australia for use in foods, and this

has created an increasing interest in the investigation of food applications for this grain.

This paper reports the findings from an analysis of the carbohydrate composition of three recently developed cultivars, selected as being most suitable for use in food applications. These results have direct implications for the production of dietary fibre fractions from the processed grain. To date only data on the alkaloid content, proximate and mineral analyses and anti-nutrient content for these cultivars is available (Harris D J pers comm 1992). These results are documented in the Australian grain legume database (Horton *et al* 1990).

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EXPERIMENTAL

Plant materials

Three cultivars of *L. angustifolius* (Gungurru, Yorrel and Danja) were obtained from the Department of Agriculture, Western Australia, from the 1987 harvest. These cultivars (Danja, released 1986, derived from crosses of P20689 wild type, Uniharvest/Mari/Unicrop; Gungurru, released 1988, derived from cross of Illyarrie/wild type P22872; Yorrell, released 1989, derived from cross of Illyarrie/wild type P22750) were selected for their low level of alkaloids (less than 0.2 g kg⁻¹ dry weight of seed), disease resistance and good yields. The hull (seed coat) was pale coloured in Danja but covered with dark brown marbling in the other two cultivars.

Lupin seeds were separated into cotyledons and hulls using a Ripple Mill (Pellet Technology Australia Pty Ltd, Summer Hill, NSW, Australia) and a fractionating aspirator. Contaminating material was manually removed. Both cotyledons and hulls were milled in dry ice using a Retsch ultra centrifugal mill, Type ZM 1, fitted with a 0.5 mm screen. Twenty seeds were dissected manually to obtain values for the proportion of hull and cotyledon in the seed.

Chemical analysis

All analyses were carried out on duplicate samples of the milled seed material.

Proximate analysis

Moisture was determined by drying the sample either in an air oven at 105°C for 16 h or in a vacuum oven at 70°C for 2 h. Total ash was determined by the method of Egan *et al* (1981). Nitrogen was measured with a Perkin Elmer 240 elemental analyser. Samples were analysed for their lipid content according to the method of Theander and Westerlund (1986).

Carbohydrate analysis

Total, insoluble and soluble non-starch polysaccharides (NSP) were analysed according to the method of Englyst and Cummings (1988) with the following modifications. After dimethyl sulphoxide treatment, enzymic hydrolysis of starch and protein was carried out using the procedure described in AACC (1990) method. Procedures for the determination of starch and Klason lignin and for the preparation of insoluble and soluble dietary fibre following enzyme treatments were those described by Theander and Westerlund (1986). Cellulose content was calculated from the difference between glucose yields from Saeman and 2M trifluoroacetic acid hydrolyses, determined as the alditol acetate derivatives by gas-liquid chromatography (Blakeney *et al* 1983). Oligosaccharides and soluble sugars were extracted with 70% ethanol (Saini and Gladstones 1986) and analysed by HPLC

using a refractive index detector and a reversed-phase C18 5 µm column (Waters) in a radial compression unit (Waters RCM-100) at room temperature, with a flow rate of 0.8 ml min⁻¹ and water as the eluant. The column was calibrated using sucrose, raffinose and stachyose standards. In this system verbascose and raffinose coeluted (Cheung P C K and Evans A J unpublished). Uronic acids were determined according to the method of Blumenkrantz and Asboe-Hansen (1973) using either *ortho*-hydroxy diphenyl or *meta*-hydroxy diphenyl.

Dietary fibre

Total and soluble dietary fibre was determined according to the gravimetric procedure of Prosky *et al* (1988).

Extraction of polysaccharides with chelating agents

Finely ground 70% aqueous ethanol extracted and defatted (with light petroleum spirit and acetone washed) hull samples (1 g), were extracted twice with 50 ml volumes of a 10 g litre⁻¹ ammonium oxalate solution pH 5.0 at 80°C for 1 h with occasional shaking. Milled, 70% ethanol extracted, defatted and starch-extracted cotyledon samples (1 g) were extracted twice with 100 ml volumes of 20 g litre⁻¹ EDTA (pH 5.0) at 85°C. The extracts were pooled, concentrated by ultrafiltration, dialysed and freeze-dried for measurement of NSP and chromatographic analysis.

Chromatography of soluble polysaccharide extract from hulls

The molecular weight of the ammonium oxalate extract from hulls was determined by high-performance gel-permeation chromatography. The sample was applied to the column as a 1% solution in 10 mM ammonium acetate. A TSK-G6000PW column (Toyo Soda Co, Ltd, Tokyo, Japan), of dimensions 600 × 7.5 mm, was used at ambient temperature, with a flow rate of 0.6 ml min⁻¹. Shodex pullulan standards P-82 (Showa Denko K. K., Tokyo, Japan) were used to calibrate the column.

RESULTS AND DISCUSSION

The chemical compositions of the three cultivars of *L. angustifolius*—Gungurru, Yorrel and Danja—are compared in Table 1. Only minor differences were observed. The similarities in composition may reflect the use of some common parental lines in their development (Ayling and Johnston 1988), although there are variations between these cultivars in plant morphology and soil and growing requirements.

The seed coat or hull of these cultivars is relatively robust representing ~ 25% of the wet weight of seed. The radical remains attached to the hull after separation by milling or dissection and constitutes 12% of this fraction (ranges for cultivars, 12.7–13.1%) thereby contributing to its chemical composition. Previous values for the weight of the hull expressed as a proportion of the

TABLE 1
Composition of cotyledons and hulls from three cultivars of *Lupinus angustifolius* (g kg⁻¹ dry weight)

	Cotyledons			Hulls		
	Gungurru	Yorrel	Danja	Gungurru	Yorrel	Danja
Proportion of seed ^a	741	759	756	259	241	244
Moisture	103	99	105	95	91	96
Ash	33	27	28	29	26	29
Nitrogen × 6.25	410	444	421	66	53	60
Lipid	86	87	84	12	12	18
NSP ^b						
Total	294	293	314	856	885	891
Soluble	46	43	44	50	49	50
Starch	6	6	7	4	3	4
Cellulose	17	8	13	494	517	510
Klason lignin	8	9	9	15	12	13
Sucrose	38	31	35	10	89	14
Oligosaccharides	77	80	74	4	3	4
Raffinose ^c	31	29	30	4	3	4
Stachyose	46	51	44	< 1	< 1	< 1
Dietary fibre ^d						
Total	285	272	310	880	901	905
Soluble	31	33	30	38	32	31

^a Calculated on a wet weight basis.

^b NSP—non-starch polysaccharide, see 'Experimental' for procedure.

^c Includes verbascose.

^d Determined by gravimetric method, see 'Experimental'.

seed, ranged, independently of seed size, from 12.8 to 31.1% for various *Lupinus* spp. (Brillouet and Riochet 1983) with a similar value being reported for *L. angustifolius* as that reported here. The analysed cultivars are therefore typical of *L. angustifolius* in having a small to average seed size with a substantial hull component. In agreement with previous studies (Bailey *et al* 1974; Harris and Jago 1985) the hull has a low content of protein and lipid and consists predominantly of structural polysaccharide. Measurements of total polysaccharide, determined as dietary fibre by the gravimetric method (range 880–905 g kg⁻¹ dry weight of hulls) or by the chemical method (NSP + Klason lignin, range 871–904 g kg⁻¹ dry weight of hulls), was in good agreement with previously published work (Bailey *et al* 1974). Only a minor proportion of the dietary fibre or NSP is soluble. Sucrose and oligosaccharides are present in only trace amounts. These results were at variance with those of Saini and Gladstones (1986) who reported values of 88–96 g kg⁻¹ dry weight for total soluble sugars extractable from hulls of three cultivars of *L. angustifolius*, with stachyose and sucrose being the predominant sugars. Cellulose is the major constituent of the structural polysaccharide of the hull of these and other lupins (Bailey *et al* 1974). In general, the results from this study and others (Bailey *et al* 1974; Brillouet and Riochet

1983; Harris and Jago 1985) indicate that hull polysaccharides are poorly lignified.

The monosaccharide composition of the total, insoluble and soluble NSP from hulls is given in Table 2. As only approx 5% of the hull NSP is soluble, the compositions of total and insoluble NSP are very similar. The cellulose content of hulls accounts for practically all the glucose present. The proportion of total NSP occurring as cellulose was 57.6, 58.4 and 57.3%, respectively, for the cultivars Gungurru, Yorrel and Danja, and consequently glucose is the most abundant constituent sugar in hull NSP. The other main sugars present were xylose, uronic acids and arabinose. This composition, which is similar to results previously reported for this and other lupin species (Brillouet and Riochet 1983), suggests that the other polysaccharides in the hull are mainly arabinoxylan hemicelluloses and pectic polysaccharides rich in uronic acids. The soluble hull material is strikingly different in composition. It could be inferred from its composition that it contains a large proportion of a galactomannan, a presumed minor hull constituent, and lesser proportions of hull pectins and hemicellulose type polymers.

Cotyledons of all cultivars had relatively high protein contents (> 400 g kg⁻¹ dry weight) and relatively low lipid contents (~ 85 g kg⁻¹ dry weight) falling within the

TABLE 2

Comparison of total, insoluble and soluble non-starch polysaccharides from hulls of three cultivars of *Lupinus angustifolius*

	Monosaccharide composition (%) ^a								
	Gungurru			Yorrel			Danja		
	Total	Insoluble	Soluble	Total	Insoluble	Soluble	Total	Insoluble	Soluble
Rhamnose	—	—	1.4	—	—	1.2	—	—	1.2
Arabinose	9.0	8.6	11.5	8.0	8.5	11.5	9.2	9.0	12.0
Xylose	14.7	12.6	40.4	15.2	12.8	41.3	14.9	12.2	41.9
Mannose	2.5	1.8	13.3	2.7	1.7	14.2	2.4	1.5	14.2
Galactose	2.2	1.4	9.9	2.2	1.4	10.1	2.2	1.5	10.0
Glucose	59.6	62.7	4.0	59.7	63.0	4.1	58.6	62.9	4.0
Uronic acids	12.0	12.9	19.4	12.2	12.6	17.6	12.8	13.0	16.6

^a Expressed as a percentage of the total polysaccharide sugars.

TABLE 3

Comparison of total, insoluble and soluble non-starch polysaccharides from cotyledons of three cultivars of *Lupinus angustifolius*

	Monosaccharide composition (%) ^a								
	Gungurru			Yorrel			Danja		
	Total	Insoluble	Soluble	Total	Insoluble	Soluble	Total	Insoluble	Soluble
Rhamnose	2.3	3.3	3.5	3.0	2.7	3.7	2.6	2.7	3.9
Arabinose	12.7	14.5	10.7	13.5	14.2	9.8	12.9	12.8	9.6
Xylose	2.4	3.2	2.4	2.3	3.0	1.7	2.2	3.2	1.6
Mannose	0.9	0.6	3.7	0.4	0.2	3.5	0.8	—	3.4
Galactose	66.5	63.3	65.9	67.0	65.7	68.6	67.5	64.8	68.5
Glucose	4.9	5.1	2.8	5.1	4.1	3.3	4.9	4.4	2.7
Uronic acids	10.3	10.0	10.9	9.7	10.1	9.5	9.6	12.2	10.3

^a Expressed as a percentage of the total polysaccharide sugars.

range of values reported previously for cultivars of *L. angustifolius* from Western Australia (Harris and Jago 1985). However, total dietary fibre values, as measured by the gravimetric (range 272–310 g kg⁻¹ dry weight) or chemical method (NSP+Klason lignin range 302–323 g kg⁻¹ dry weight) were much higher than had been observed previously for *L. angustifolius* (Brillouet and Riochet 1983). Within the range of values for cotyledon cell wall material values of 75–321 g kg⁻¹ dry weight for lupin species (Brillouet and Riochet 1983) only one species, *L. pilosus*, had values similar to those obtained for the cultivars in our study. Only a small proportion of the cotyledon dietary fibre was present in a soluble form. As there is little starch present in the cotyledons, the major polysaccharide reserve is presumed to be material accumulating in the cell wall, as previously described in other lupins (Crawshaw and Reid 1984). This would account for the low content of structural components, such as cellulose and lignin, in the cotyledons.

Sucrose was the only soluble sugar measured with values ranging from 31–38 g kg⁻¹ dry weight. Saini and Gladstones (1986) obtained values of 40–82 g kg⁻¹ from

a range of cultivars of *L. angustifolius* which were generally higher than their results for other lupin species. The major oligosaccharides in *L. angustifolius* are stachyose, verbascose and raffinose with their respective order indicating their relative abundance (Macrae and Zand-Moghaddam 1978; Quemener 1988; Trugo *et al* 1988). With the HPLC method used in our study verbascose coelutes with raffinose. Stachyose values for the three cultivars ranged from 44 to 51 g kg⁻¹ dry weight and raffinose plus verbascose values ranged from 29 to 31 g kg⁻¹ dry weight, with overall the total oligosaccharide content ranging from 74 to 80 g kg⁻¹ dry weight. HPLC analysis of a recent sample of the cultivar Gungurru, using a CarboPac column PA1 (4 × 250 mm, Dionex, California, USA), which separates all oligosaccharides gave values of 38, 22 and 9 g kg⁻¹ dry weight for stachyose, verbascose and raffinose respectively (Cheung P C K and Evans A J unpublished). In general these results are similar to those reported previously (Macrae and Zand-Moghaddam 1978; Quemener 1988; Trugo *et al* 1988). Higher values for total oligosaccharides in lupins have been reported by Saini

TABLE 4

Comparison of soluble polysaccharide extracted with chelating agents from hulls and cotyledons of *Lupinus angustifolius* cv Gungurru

	Hull	Cotyledon
Extraction Conditions	1% ammonium oxalate, pH 5.0, 80°C	2% EDTA, pH 5.0, 85°C
Yield (g kg ⁻¹) ^a	136	175
Monosaccharide composition (%) ^b		
Rhamnose	3.5	2.0
Arabinose	30.7	11.9
Xylose	16.0	1.5
Mannose	6.2	—
Galactose	7.1	79.1
Glucose	0.8	0.5
Uronic Acids	35.8	5.0

^a Expressed as g kg⁻¹ dry weight of hull or cotyledons.

^b Expressed as a percentage of the total polysaccharide sugars.

and Gladstones (1986) which may be attributable to the analytical procedures used.

The monosaccharide composition of the total cotyledon NSP (Table 3) is similar to that reported elsewhere for *L. angustifolius* (Brillouet and Riochet 1983) but molar ratios of galactose to arabinose varied from 4.12 to 4.36 being higher than values primarily obtained for this species (Brillouet and Riochet 1983). As has been previously noted (Brillouet and Riochet 1983) high values for this ratio were associated with a high cell wall content. The molar ratio of uronic acids to arabinose was similar to that reported previously for *L. angustifolius* (Brillouet and Riochet 1983). The soluble NSP was similar in composition to the total or insoluble material (Table 3), although glucose and arabinose were reduced in concentration and mannose increased. As most of the glucose occurs as cellulose, the result for glucose is expected.

Significant amounts of soluble polysaccharide could be extracted from hull or cotyledon material using chelating agents (Table 4). The yield from hulls was 136 g kg⁻¹ dry weight for a material rich in uronic acids, arabinose and xylose and substantially different in composition from the parent and water soluble material (Table 2). Bailey *et al* (1974) also reported extracting a polysaccharide rich in uronic acids from the hulls of *L. angustifolius* by using oxalate. Gel permeation chromatography indicated the presence of a very broad peak of molecular weight of 600×10^3 . A much higher yield of EDTA-soluble polysaccharide material (when considered as a proportion of the total cotyledon polysaccharide) could be obtained from the cotyledons using a chelating agent (Table 4). The monosaccharide composition of this extract was quite similar in composition to the total or soluble polysaccharide from the cotyledons (Table 3).

The cotyledons of some species of lupins have already been shown to possess well-developed and thickened cell walls which contain a predominance of reserve polysaccharide material utilised by the germinating seed (Crawshaw and Reid 1984; Parker 1984). It would appear that this morphological feature which may have some benefit for the germination and early growth of seeds in a more arid environment, has been further developed by the selection processes used in producing the cultivars, Gungurru, Danja and Yorrel, analysed in this study. Thus, these cultivars contain a significantly greater amount of cotyledon cell wall material than has been reported previously for *L. angustifolius* and most other lupin species. The combination of high protein and cell wall material levels in these cultivars (700–750 g kg⁻¹ dry weight of cotyledons) consequently means that the lipid content is relatively low. In processing these cultivars for the production of foods or food ingredients, the two major products will be protein and cell wall material or dietary fibre. The findings from this study indicate that the cell wall material content of the cultivars investigated is sufficiently high to regard them as a potentially important source of dietary fibre. Work is now in progress to isolate, characterise and evaluate dietary fibre materials extracted from these cultivars.

ACKNOWLEDGEMENTS

This work was supported by the Grain Legumes Research Council of Australia.

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