Composition of *Lupinus albus*[1]

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ABSTRACT

Analysis of *Lupinus albus* L.2043N is described. Total oligosaccharide content determined with high pH anionic chromatography, high-pressure liquid chromatography, and pulsed amperometric detection (HPAC-HPLC-PAD) yielded 5.3%. The most abundant oligosaccharide was stachyose (2.8%), followed by sucrose (1.8%), raffinose (0.4%), and verbascose (0.3%). Verbascose plus stachyose (3.1%) content was lower than the literature reports for soybeans (4.6%). *Lupinus albus* L.2043N had a lower phytic acid content (0.03%) than the reported values for soybeans (1.54%). Uronic acid (12%) and total dietary fiber (34.2%) content were three times higher in the lupin samples than in hard red spring wheat and oats. No free monosaccharides were detected, except for a trace of fructose. Proximate analysis showed that *L. albus* L.2043N was higher in protein (38%) and lower in starch (3%) than other common legumes. Ash content (4%) was similar to other lupin species and similar to soybeans. Oil content (10%) was lower than in soybeans. The total carotenoid content of the whole grain was 36 ppm.

Lupin is a legume used as human food and animal feed since early Roman times. Over 200 lupin species are grown world wide. Lupin can tolerate frost, drought, and poor soils. In the Northern Plains of United States, lupin can be grown in North and South Dakota, Wisconsin, and Minnesota. However, utilization of lupin since its domestication has been limited, and this may be partly due to its alkaloid content and low yield. Breeding programs have produced "sweet" varieties with as low as 0.002% alkaloid content, which makes them safe for human consumption (Culvenor and Petterson 1986). The alkaloids are reduced by soaking and washing the seeds. Hemicellulase and cellulase have facilitated the removal of alkaloids in lupins (Santana et al 1990). The yield of lupin has increased, especially in Australia, Chile, and the United States as a result of breeding programs. The agronomical benefits of growing lupin include its ability to fix nitrogen and to grow in areas where soybeans cannot be grown (Rahman and Gladstones 1987).

The genus *Lupinus* typically contains 36–52% protein, 5–20% oil, and 30–40% fiber (Gross et al 1988, Petterson and Mackintosh 1994). The variation in composition is due to genetic and environmental differences (Hill 1986).

Lupin fatty acid composition is mainly unsaturated with oleic and linoleic acid comprising 86% of the oil (Williams and McGibbon 1980). The alkaloid content of lupin varies among cultivars, soil type, and growing season (Gladstones 1970). Lupin is lower in antinutritional factors than soybeans. Lupin seeds or meal would not need to be heat-treated since trypsin inhibitors and hemaglutinnins are practically absent (Hill 1977, 1986; Schoeneberger et al 1983; Matthews 1989). Lupin is low in sodium and calcium (Rahman and Gladstones 1987) compared to other legumes. It is high in carotenoids and zeaxanthin, which give the cotyledon its bright yellow color (Entisar and Hudson 1979). Lupin is low in starch (2.3%), while other common legumes contain up to 50% starch (Cerning-Beroad and Filiatre 1976).

The polysaccharide content of lupin cotyledon is mainly galactan, and the hull is mainly cellulose or hemicellulose. The lupin hull comprises 25% of the grain and is low in lignin (Jean-Marc and Carre 1989).

Lupin protein has good functional properties, i.e., emulsifying power, binding, and foaming properties (Edwin 1974). Lupin protein is deficient in sulfur-containing amino acids. The protein efficiency ratio (PER) of whole lupin and dehulled seeds has been reported at 1.29–1.39 and 1.43–1.83, respectively (Edwin 1974). After DL-methionine supplementation (3 g/kg of diet), the PER increased to 2.40–2.56 and 2.65–2.78, for whole and dehulled lupin, respectively (Edwin 1974). Romana (1984) reported a biological value of diets, containing primarily *L. mutabilis* flour and consumed by children, of 61.3 and 84.8% unsupplemented and supplemented with 0.2% methionine, respectively. These values were very similar to those reported by Savage et al (1984).

Lupin could be used as a source of protein or fiber and for supplementation in existing or new products. Lupin can also be used in breadmaking, biscuits, pasta products, and a variety of other food products. For a review on lupin utilization in human foods see Hill (1986).

The interest in utilization of lupin has increased worldwide, with Australia emerging as a major advocate. New markets in Australia for lupins being considered for human foods as well as animal feed are intensive fisheries, poultry, dairy, and beef cattle (Hough and Jacobs 1994, Jenkins et al 1994, Kyle 1994, van Barneveld and Hughes 1994). There are few studies on the analysis of lupins adapted to the United States environment. The objective of this research was to analyze the composition of the elite *Lupinus albus* cutlivar obtained from the United States breeding program.

MATERIALS AND METHODS

Chemicals

Hydrochloric acid, Na_2SO_4, $FeCl_3 \cdot 6H_2O$, ammonium molybdate, Fiske-Subbarow reagent, sodium phosphate, heat-stable α-amylase, pronase, Carrez solutions 1 and 2, α-amylase E.C. 3.2.1.1, pullulanase E.C. 3.2.1.41, and amyloglucosidase E.C. 3.2.1.3 were obtained from Sigma Chemical Co. (St. Louis, MO). Sodium chloride-boric acid mixture (4:18, w/w) and 3,5-dimethylphenol were used.

Seed Samples

Lupinus albus L.2043N seed, a low alkaloid (0.009%) line, was donated by Gene Aksland (Resource Seeds Inc., Gilroy, CA). The seeds were grown in 1992 and stored at −7°C until analysis. Samples of hard red spring wheat cultivar Grandin and oats culti-

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var Dumont from the crop year 1994 were obtained from the Cereal Science Department.

Determination of Uronic Acid

Lupin samples were treated according to the method of Englyst et al (1982). After the hydrolysis step, aliquots of hydrolyzate were removed, and uronic acid content was determined according to the method of Scott (1979) with the following modifications: 1) an aliquot (0.3 ml) was diluted with 6 ml of water; 2) an aliquot (0.3 ml) of a solution containing 2 g of NaCl + 3 g of boric acid/100 ml was added; and 3) 0.2 ml of 3,5-dimethylphenol solution (0.1 g in 100 ml glacial acetic acid) was used. A standard curve was constructed using 20, 40, 60, 80, and 100 µg of galacturonic acid.

Total Dietary, Soluble, and Insoluble Fiber

Total dietary, soluble, and insoluble fiber were determined by the AOAC method 985.29 (1990), AACC method 32-07 (1983), and the enzymatic-gravimetric AOAC method 991.43 using MES-TRIS buffer (Sullivan and Carpenter 1993), respectively.

Determination of Phytic Acid

Phytic acid was analyzed according to Thompson and Erdman (1982) as ferric phytate precipitate using 4 hr and 115°C during the digestion step. The specific procedure steps were: 1) 100 ml of 1.2% HCl were added to a 2-g lupin flour sample, and the solution was shaken for 2 hr with a mechanical shaker (Lab-Line Instrument, Inc., model 3528-5 Melrose Park, IL) with speed control set at 250 rpm; 2) a 10-ml aliquot of filtrate was mixed with 12 ml of $FeCl_3$ (2 g of $FeCl_3$ + 16.3 ml of conc. HCl, diluted to 1 L), heated for 4 hr at 115°C, and cooled to room temperature at 25°C; 3) the suspension was centrifuged at 2,060 × *g* for 10 min and filtered through Whatman paper #1; 4) the filtrate was diluted to 50 ml, and 2 ml were withdrawn for analysis of soluble phosphorus, as described by Bartlett (1959) using ammonium molybdate and the Fiske and Subbarrow reagent; 5) absorbance was read at 810 nm; 6) 1 ml was taken from the first filtrate, diluted to 200 ml, and 2 ml were analyzed for total phosphorus, and absorbance was read at 810 nm. A standard curve was constructed using KH_2PO_4 in 10*N* H_2SO_4 at concentrations of 0.02, 0.05, 0.1, 0.15, and 0.2 µg.

Determination of Oligosaccharides and Monosaccharides

Oligosaccharide extraction was based in the procedure of Macrae and Zand-Moghaddam (1978), with the following specific steps. Lupin flour was defatted with hexane for 3 hr. Two grams of defatted flour were refluxed in 60 ml of water and methanol (6:4, v/v) at 100°C for 25 min, cooled, and centrifuged at 2,060 × *g* for 5 min. The residue was extracted twice and once with boiling distilled water. The combined supernatants were dried in a vacuum oven at 45°C and redissolved in 15 ml of water. The suspension was treated with 2 ml of Carrez 1 and 2 solutions to precipitate the proteins. The solution was centrifuged at 2,060 × *g* for 10 min, and the supernatant was filtered and aliquots used for carbohydrate analysis using a high pH anion exchange chromatography and high-performance liquid chromatography (HPAC-HPLC) system.

Total monosaccharides were extracted according to the method of Ponte et al (1969), with the following modifications. Chloroform and methanol (1:1, v/v) was added to lupin flour at a 1:4 ratio (w/v). The suspension was mechanically shaken for 30 min and centrifuged at 1,970 × *g* for 15 min. The top layer was removed, and the residue was extracted twice with 4 ml of methanol and water (1:1, v/v) and centrifuged at 1,970 × *g* for 10 min. The supernatants were combined and filtered. An aliquot (25 µl) was injected onto the Dionex HPAC column.

HPAC-HPLC Analyses

A Dionex TN 20 HPAC-HPLC System (Sunnyvalley, CA) with pulsed amperometric detector equipped with a gold working electrode with 3000 nAmps sensitivity was used. Pulse settings with (E_1–E_3) potentials and (t_1–t_3) durations used were E_1 = 0.05V (t_1 = 480 msec), E_2 = 0.6V (t_2 = 120 msec), and E_3 = –0.6V (t_3 = 60 msec). A CarboPac PA1 (4 × 250 mm) column (P/N 35391) and a CarboPac PA1 guard (3 × 25 mm) column (P/N 37141) were used. Sodium hydroxide, 150 Mm, at a flow rate of 1.0 ml/min, was used as the eluant. The isochratic runtime was 25 min. Individual standard curves of glucose, galactose, mannose, rhamnose, sucrose, raffinose, stachyose, and verbascose were constructed, using 0.05, 0.1, 0.5, 1.0, 1.5, and 2.0 µg of each sugar.

Total Starch

Total starch was determined according to the method of McCleary et al (1992), using a MegaZyme Kit (Warriewood, Sydney, Australia).

Total Carbohydrates

The phenol-sulfuric acid method described by Dubois et al (1956) was used. Five grams of lupin flour (40 mesh sieve) was suspended in 100 ml of 0.1*M* phosphate buffer, pH 6.5. The suspension was diluted 1:100 with distilled water, and aliquots (1.0 ml) were used for analysis. Anhydrous glucose at 10, 20, 30, 50, 100, and 150 µg/ml was used to prepare a standard curve.

Proximate Analysis

Moisture, protein, ash, and oil were determined according to the AACC (1983) standard methods 44-15, 46-10, 08-01, and 30-25, respectively. Protein content was calculated as N × 6.25, and a mixture of potassium sulfate, copper sulfate, and titanium oxide (20.9 g of K_2SO_4 + 0.0125 g of $CuSO_4$ + 0.75 g of TiO_2) was used as a catalyst. Oil was extracted overnight in a Soxhlet extractor with hexane.

Color

The color of the whole seeds, hulls, and cotyledons and whole flour was analyzed using a Minolta Color Difference Meter, model CR-310 (Minolta, Japan) equipped with a 50-mm diameter measuring head. Readings of *L* (measure of light reflectance), *a*

TABLE I
Uronic Acid and Total Dietary Fiber (TDF) Content in *Lupinus albus*, Hard Red Spring (HRS) Wheat, and Oats[a]

Grain	Uronic Acid, %[b]	TDF, %
L. albus	12.0 ± 0.02	34.2 ± 1.0
HRS (cv. Grandin)	3.4 ± 0.02	11.7 ± 0.7
Oats (cv. Dumont)	3.5 ± 0.02	12.3 ± 1.5

[a] Mean ± standard error; $n = 2$, expressed on a dry weight basis.
[b] Regression analysis equation used to determine the uronic acid content: µg of uronic acid = 112.2 Y – 2.9, where Y = absorbance difference from $A_{450} - A_{400}$.

TABLE II
Comparison of Phytic Acid and Starch Content of *Lupinus albus*[a] and Other Legumes

	Phytic Acid, %	Starch, %
L. albus	0.03 ± 0.01	3.0 ± 0.11
Glycine max, soybeans[b]	1.54	···
Tofu[b]	1.96	···
Phaseolus vulgaris, navy bean[c]	···	51
P. aureus, mung bean[c]	···	50

[a] Mean ± standard error, $n = 2$, expressed on a dry weight basis.
[b] Thompson and Erdman (1982).
[c] Naivikul and D'Appolonia (1978).

(represents red when positive and green when negative), and *b* (yellowness, represents yellow when positive and blue when negative) values were converted to corresponding Hunter Color Difference Meter values. Lupin whole seeds were milled into flour using a Labconco mill (Laboratories Construction, Kansas City, MO) adjusted to give coarse particles, followed by a Falling Number Mill (Falling Number, Reno, NV) with 1-mm screen. The flour color values were measured immediately after grinding. The hulls and cotyledons were obtained by a short grinding step in a blender followed by screening the hulls and selecting complete dehulled cotyledon samples. Two independent samples were analyzed and two readings from each sample were recorded.

Total Carotenoids

Total carotenoids were determined in triplicate in whole grain ground to pass 60-mesh size using AOAC (1980) method 14.045.

RESULTS AND DISCUSSION

Uronic Acid

L. albus L.2043N contained about three times more uronic acid than hard red spring (HRS) cv. Grandin and oats cv. Dumont (Table I). Uronic acid content expressed as galacturonic acid equivalent has been reported to be a good indicator of pectin material (Lopez et al 1990). Raymond et al (1974) reported that lupin seeds contained higher pectin substances than other legumes. Kikuchi and Ishil (1971) reported that the insoluble carbohydrate fraction of soybean contained 4% pectin substances.

Total Dietary (TDF), Soluble, and Insoluble Fiber

The *L. albus* L.2043N contained ≈2.8–2.9 times the amount of TDF found in HRS and oats (Table I). The polysaccharide composition of lupin huli and cotyledons was reported by Mohamed and Rayas-Duarte (1995). Lupin cotyledons contained 21.5 and 2.2% insoluble and soluble fiber, respectively, while the hull contained 86.2 and 1% insoluble and soluble fiber, respectively. The composition of "dietary fiber products" extracted from *L. angustifolius* from the hull or cotyledon mainly differed in the galactose content; the main component in the cotyledons was galactose and glucose in the hull (Evans and Cheung 1991). The same authors reported that there was approximately twice the water holding capacity in the cotyledon as in the hull fiber.

Phytic Acid

A comparison of the phytic acid content of the lupin seeds with literature-reported values of soybean and tofu are presented in Table II. Trugo et al (1993) reported that lupin varieties grown in South America contain 0.4–1.2% phytic acid. These values were similar to those of lentil beans and peas (Donangelo et al 1986), but lower than soybeans (Thompson and Erdman 1982). Phytic acid forms an insoluble compound with minerals, especially with zinc (Sandstrom 1988). The phytate to mineral molar ratio rather than the absolute phytate levels has been proposed as an indicator of mineral bioavailability (Ellis et al 1987). The phytate to zinc ratio of lupin grown in South America was 17, higher than that grown in North America (10.9), while in soybean, the ratio was 25 (Petterson et al 1990). Some lupin varieties have been reported to contain phytate-to-calcium ratio higher than 0.2 (Morris and Ellis 1985), which can produce a significant decrease of calcium availability.

Total Starch

Lupinus albus L.2043N contained 3% starch (Table II), a significantly lower content when compared with the values reported for other legumes. Lupin cotyledons have been reported to store reserve galactose-rich polysaccharides in the cell wall that are depleted during germination (Parker 1984, Trugo and Almeida 1988).

Oligosaccharides

Oligosaccharides extracted from lupin were analyzed with HPAC-HPLC-PAD, which allowed the detection of carbohydrates with a range of 1–19 monosaccharide units. Unlike the refractive index detector, peptides and amino acids did not interfere with pulsed amperometric detection. Stachyose (2.8%) was the most abundant oligosaccharide in lupin, followed by sucrose (1.8%), raffinose (0.4%), and verbascose (0.3%) (Table III).

Stachyose and verbascose are considered to have the greatest influence on gas production when oligosaccharides are hydrolyzed by intestinal bacteria. A comparison of stachyose + verbascose content in the lupin variety *L. albus* L.2043N with other lupin species and soybeans (Table IV) suggested that a lower flatulence factor would be expected in the former. This may be an advantage of *L. albus* that could be exploited to enhance its food use (Macrae and Zand-Moghaddam 1978).

The amount of oligosaccahrides in lupin is influenced by the climate and the soil. *Lupinus albus* (cv. Hamburg) grown in Germany and *L. albus* (cv. Ultra) grown in South America contained a higher oligosaccarides content than the *L. albus* L.2043N (Table V). Germination and lactic acid bacteria fermentation are among the proposed methods to lower oligosaccharides content in lupin (Trugo and Almeida 1988). Sucrose was found (5%) in *L. albus* grown in Argentina (Trugo and Almeida 1988).

Monosaccharides

Only a trace of fructose was detected in the lupin sample reported here. Horbowicz and Obendorf (1994) reviewed and surveyed 19 species of seeds, including lupin, at mature dry state and found only a trace of glucose or fructose.

TABLE III
Oligosaccharide Composition of *Lupinus albus* Whole Seeds[a,b]

Sugar	Percentage
Sucrose	1.8 ± 0.10
Raffinose	0.4 ± 0.01
Stachyose	2.8 ± 0.01
Verbascose	0.3 ± 0.01
Total	5.3 ± 0.10

[a] Mean ± standard error; $n = 2$, expressed on a dry weight basis.
[b] Determined by high pH anionic chromatography—high-pressure liquid chromatography and pulsed amperometric detection (HPAC-HPLC-PAD).

TABLE IV
Comparison of Verbascose + Stachyose in Two Lupin Species and Soybean Seeds

Sample	Stachyose + Verbascose, %
Lupinus albus[a]	3.1 ± 0.1
L. mutabilis[b]	10.0
Glycine max, soybeans[b]	4.6

[a] Mean ± standard error; $n = 2$, expressed on dry weight basis.
[b] Macrae and Zand-Moghaddam (1978).

TABLE V
Total Oligosaccharides Content in Three *Lupinus albus* Cultivars

Cultivar	Growing Location	Oligosaccharides, %
L.2043N[a]	California	5.3 ± 0.10
Ultra[b]	Argentina	8.6
Hamburg[c]	Germany	10.3

[a] Mean ± standard error, $n = 2$, expressed on a dry weight basis.
[b] Saini et al (1986).
[c] Trugo and Almeida (1988).

TABLE VI
Chemical Composition of *Lupinus albus* and Other Legumes

Legume	Protein %[a]	Oil %	Ash %	Starch %	Total CHO %[b]
L. albus[c]	38.0 ± 0.8	10.0 ± 0.3	4.0 ± 0.1	3.0 ± 0.1	48.0 ± 0.2
L. angustifolius[d]	34.8	19.0	4.4	3.3	46.0
Glycine max, soybeans[d]	34.0	17.0	4.9	51.0	...

[a] N × 6.25.
[b] Total carbohydrate.
[c] Mean ± standard error; $n = 2$, expressed on a dry weight basis.
[d] Mathews (1989). Composite from different tables.

TABLE VII
Color Scale Values of Lupin Whole Grain, Hulls, Cotyledons, and Flour

Lupin Products	Hunter Values[a]		
	L	*a*	*b*
Whole grain	61.21 ± 0.10	+2.22 ± 0.10	+11.47 ± 0.04
Hulls	65.28 ± 0.67	+1.76 ± 0.04	+13.53 ± 0.30
Cotyledons	55.19 ± 0.54	+6.12 ± 0.38	+22.44 ± 0.18
Flour[b]	82.78 ± 0.15	−1.98 ± 0.01	+21.33 ± 0.06

[a] Mean ± standard deviation; $n = 4$.
[b] Whole grain flour.

Total Carbohydrates

The total carbohydrates content of lupin seeds used in this study was 48% (Table VI). This amount is comparable to the *L. angustifolius* grown in Australia and higher than that of soybeans.

Proximate Analysis

Lupin protein content varied among cultivars, but was generally higher than other legumes (Table VI). Hove (1974) reported a range of 28.6–32.4% protein in four cultivars of *L. angustifolius* and 40% protein in one cultivar of *L. luteus*. Sathe et al (1982) reported that *L. mutabilis* (cv. H-4) protein had good water and oil absorption and gelation properties. No trypsin, chymotrypsin, and α-amylase inhibitors were detected in lupin flour or protein isolates (Sathe et al 1982).

The oil content of the cultivar reported here was lower than that of soybean. However, some lupin cultivars contained up to 25% oil (Gross et al 1988). The ash content (4%) is in the range of other lupin species and similar to that of soybeans (Table VI).

Color

The color of lupin seeds and different products is reported in Table VII. Lupin hulls with a visual (subjective) appearance of white color had high light reflectance *L* and lower red (*a*) values. Cotyledons showed the highest yellowness (*b*) and red (*a*) values. Lupin flour was characterized as bright yellow with green tones (*a* negative). The bright yellow color of the whole flour and cotyledons might have applications in substituting egg yolks or yellow dyes in food products. The white hull color might have advantages as a fiber source with reduced interference of food color and appearance. A study of the functional properties of lupin fractions is in progress in our laboratory.

Total Pigments

The red-yellow color of lupin cotyledon is a desirable factor that may play an important role in its utilization. Whole lupin flour had a total pigment content of 36 ppm, higher value than the reported (4.7 ppm) by Entisar and Hudson (1979) in *L. albus* in the United Kingdom. This value is higher than the range of 5.50–8.42 and 6.49–7.94 ppm of pigments found in Canadian durum wheats (Canadian Grain Commission 1980) and durum wheat samples with different levels of starchy kernels (Matsuo and Dexter 1980). McDonald (1979) reported the lutein (xanthophyll) content, in nine durum semolinas ranging from 2.74 to 6.24 μg/g. The major carotenoids reported by Entisar and Hudson (1979) in four lupin varieties were zeaxanthin and β-carotene; among the four varieties reported *L. mutabilis* and *L. luteus* contained higher total carotenoids amounts than *L. angustifolius* and *L. albus*. However, carotenoid analysis of new lupin cultivars are needed to compare the values with updated analysis methods in the improved lupin lines now available.

CONCLUSIONS

Lupinus albus L.2043N had lower oligosaccharides (5.3%), phytic acid (0.03%), and oil (10%) contents than values reported for other lupin varieties and legumes. The amount of uronic acid (12%) expressed as galacturonic acid, total dietary fiber (34.2%), protein (38%), and total carbohydrates (48%) was higher than in other legumes. Composition, color, and total pigments are important properties of lupins that can enhance its utilization as human food ingredient or a source of protein for in-farm feed use.

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