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Effect of germination on the oligosaccharide content of lupin species

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ABSTRACT

The effect of germination on the oligosaccharide content of two lupin species (*Lupinus albus* and *Lupinus luteus*) was determined with an extraction method, using aqueous methanol and purification through ion-exchange minicolumns. The quantification was carried out using high-performance liquid chromatography and showed that during germination of both species there was a clear reduction in the levels of raffinose family oligosaccharides.

INTRODUCTION

Legumes are well known inducers of intestinal gas (flatulence) because of the presence of oligosaccharides of the raffinose family. These sugars are characterized by the presence of α-D-galactopyranosyl residues bound to the glucose moiety of sucrose [1]. Animals and man are not able to digest such oligosaccharides because of the absence of α-1,6-galactosidase in their intestinal mucosa. Consequently the raffinose oligosaccharides pass into the colon and, as they are unable to be transported across the intestinal wall, are fermented by intestinal bacteria with considerable production of gas, mainly carbon dioxide [2]. It is clearly desirable to decrease the oligosaccharide content of legumes if they are to be most efectively exploited as inexpensive sources of protein.

A number of studies have indicated that the content of raffinose oligosaccharides in a range of legumes decreases during germination [3–5]. This could consequently increase the nutritional value of these foods, since there is evidence that protein content and quality are unaffected [6,7].

Lupin seed represents a potentially important source of protein for animal and human consumption. The utilization of this crop has been limited for some years as a result of the presence of toxic alkaloids [8], but low-alkaloid, "sweet" varieties are now available for cultivation. The present study was initiated to examine the effect on oligosaccharide content of germination of two species of lupin, *Lupinus albus* and *Lupinus luteus*.

EXPERIMENTAL

Seeds of two species of bitter lupin (*L. albus* from Badajoz and *L. luteus* from Huelva) were used in the present study. The surface of the seeds was sterilized with 6% calcium hypochlorite and washed repeatedly with deionized water prior to germination.

Chemicals

Water purified with a Milli-Q system (Millipore, Bedford, MA, USA) was used throughout.

High-performance liquid chromatography (HPLC)-grade acetonitrile and methanol were purchased from Scharlau (Barcelona, Spain). Other

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chemicals, all of analytical or reagent grade, were supplied by Prolabo, Merck or Fluka (Buchs, Switzerland).

Germination

The washed seeds were soaked in water for 3 h and germination was effected in a dark chamber at 29°C, water being added as necessary. Samples of 400 seeds were removed at 24, 48, 72, 96 and 120 h, dried and ground to pass through a 100-mesh sieve. Samples of the dry, non-germinated seeds were ground and homogenized. Chromatographic analysis was carried out four times on each sample, the germination procedure being repeated in triplicate.

Extraction

Two extraction methods were used:

(a) Finely ground material (2 g) was treated with aqueous methanol (60% methanol, 40 ml). The suspension was boiled at 92°C under reflux for 2 h, cooled and centrifuged at 700 *g* for 5 min. The supernatant was removed and the residue extracted twice more with boiling aqueous methanol and finally washed with water (40 ml). The combined extracts and washings were evaporated to dryness *in vacuo* below 50°C [9]. The residue was dissolved in water (25 ml) and an aliquot of 3.5 ml was transferred into a glass-stoppered test tube; acetonitrile (6.5 ml) was added with shaking and the mixture allowed to stand at 5°C for 1 h [10]. After filtering (Millipore, 0.45 μm), the sample (20 μl) was injected into the HPLC system.

(b) Ground material (0.5 g) was homogenized with aqueous methanol (70% methanol, 5 ml) for 1 min at room temperature. The mixture was centrifuged for 5 min (700 *g*) and the supernatant decanted. The procedure was repeated twice more and the combined supernatants evaporated *in vacuo* at 35°C. The product was dissolved in double-deionized water (1 ml) and passed through Dowex (Dow

TABLE I

RECOVERY OF OLIGOSACCHARIDES USING EXTRACTION METHODS a AND b

Oligosaccharide	Amount added (mg)	Total amount found (mg)	Recovery (%)
Method a (in 2 g of flour)			
Sucrose	0.00	29.29	–
	20.21	46.15	83.42
	40.08	69.29	99.80
	80.23	101.43	89.84
Raffinose	0.00	21.64	–
	20.14	37.15	77.01
	40.25	56.43	86.43
	80.20	98.22	95.49
Stachyose	0.00	120.36	–
	20.07	137.86	87.19
	40.06	157.15	91.84
	80.21	187.72	83.98
Method b (in 500 mg of flour)			
Sucrose	0.00	8.10	–
	5.21	13.70	107.49
	10.30	18.26	98.64
	20.18	27.32	95.24
Raffinose	0.00	2.48	–
	5.22	7.30	92.34
	10.19	11.36	87.14
	20.36	20.38	87.92
Stachyose	0.00	27.38	–
	5.04	34.06	132.54
	10.19	37.46	98.92
	20.09	45.52	90.29

Chemical) 50W-X8 (200–400 mesh) and Waters QMA minicolumns with a Supelco vacuum system (Bellefonte, PA, USA). Water (three 1-ml portions) was added to flush the columns and the combined extracts and washings were collected and injected (20 μl) into the HPLC system.

HPLC analysis

Samples (20 μl) were analysed using a Konik chromatograph fitted with an ERMA 7520 refractive index detector (Barcelona, Spain). A Spherisorb-5-NH_2 column (250 × 4.6 mm I.D.) (Teknokroma, Bellefonte, PA, USA) was employed with acetonitrile–water (1 ml/min^{-1}) in two different proportions [72:28 (v/v) in method a and 65:35 (v/v) in method b] as the mobile phase. Individual sugars were quantified by comparison with external standards of sucrose, raffinose, stachyose (Sigma, St. Louis, MO, USA) and a standard sample of verbascose that was obtained from *Vicia faba* (cv. Alameda) seeds, as described below.

Extraction of verbascose

The ground *V. faba* flour was extracted according to method b above, and the residue dissolved in water (1 ml). Individual sugars were separated by preparative thin-layer chromatography on silica gel plates (Whatman's PLK 5F, 20 × 20 cm, 1000 μm). The solvent system was butanol–acetic acid–water (12:3:5), colour development being achieved by aniline–diphenylamine–phosphoric acid [11]. The verbascose band was removed and dissolved in water and the mixture centrifuged at 700 *g* for 10 min; the supernatant was removed and the water evaporated *in vacuo*.

Statistical analysis

The data were analysed for variance using a BMDP-2V ANOVA programme (W. J. Dixon, BMDP Statistical Software, Software Release, 1988) and the mean values compared using Duncan's multiple range test.

RESULTS AND DISCUSSION

HPLC was used to quantify the individual sugars, sucrose, raffinose, stachyose and verbascose. Calibration curves were constructed for all four sugars; a linear response was evident for the range 0–5 mg/ml^{-1}, correlation coefficients being 0.99. The recoveries of oligosaccharides using methods a and b are presented in Table I. The results of this study show satisfactory recoveries using both methods, but higher values were found with method b. Because of these results, quantification of the effect of germination on oligosaccharides was carried out using method b.

Fig. 1 shows HPLC patterns for the dry (non-germinated) seeds of *L. albus* and *L. luteus*. The α-galactoside contents in these seeds before and during germination are listed in Tables II and III. In *L. albus*, stachyose was originally present in much larger amounts than raffinose or verbascose, whilst in *L. luteus* the contents of stachyose and verbascose were approximately the same and greatly in excess of the amount of raffinose. The values obtained for the dry beans agree with figures reported for these species by other authors [9,12]. Trials on animals have shown that the molecular mass of these oligosaccharides has an influence on flatus formation. Stachyose, the tetrasaccharide, and verbascose, the pentasaccharide, have marked effects, while raffinose, the trisaccharide, has an insignificant effect [9].

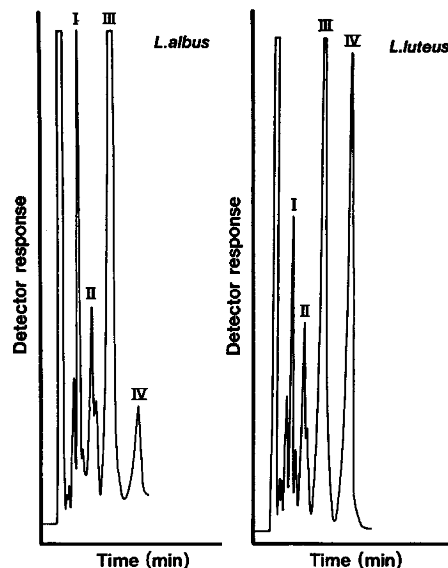

Fig. 1. HPLC patterns of α-galactosides from an aqueous extract for dry (non-germinated) seeds of *L. albus* and *L. luteus* oligosaccharides numbered as sucrose (I), raffinose (II), stachyose (III) and verbascose (IV). A Spherisorb-5-NH_2 column (250 × 4.6 mm I.D.) was employed with 65:35 (v/v) acetonitrile–water (1 ml/min^{-1}) as the mobile phase.