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Raffinose family oligosaccharides and sucrose contents in 13 Spanish lupin cultivars

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Abstract

The contents of raffinose family oligosaccharides (RFOs) and sucrose in 13 Spanish cultivars of lupin (*Lupinus albus*, *Lupinus luteus* and *Lupinus angustifolius*) were studied, with the aim of selecting those with highest amounts of these oligosaccharides in order to obtain pure RFOs for use as ingredients in functional foods. The levels of sucrose and RFOs (raffinose, stachyose and verbascose) were determined using high perfornnance liquid chromatography (HPLC). There were large variations in the levels of individual RFOs between lupin cultivars. *L. albus* seeds were characterised by the lowest verbascose content (∼0.4%), *L. luteus* seeds by the highest contents of stachyose (∼7.4%) and verbascose (∼3.1%) and the lowest of sucrose (∼1.2%),and *L. angustifolius* seeds by the highest sucrose (∼3.4%) and the lowest stachyose (∼4.6%) contents. Furthermore, there was a wide variation in total α-galactosides between species, with a remarkably high content found in *L. luteus* (9.5–12.3%) which was about twice that of other lupin cultivars. For this reason, *L. luteus* seeds are the best choice for obtaining pure RFOs for use as prebiotics in functional foods.

Keywords: Raffinose family oligosaccharides; Lupin; Raffinose; Stachyose; Verbascose

1. Introduction

α-Galactosides, called also galacto-oligosaccharides and raffinose family oligosaccharides (RFOs) are widely distributed in the plant kingdom. Large amounts of RFOs occur in the generative parts of higher plants where they perform protective physiological functions (Dey, 1985; Horbowicz & Obendorf, 1994; Kuo, Van Middlesworth, & Wolf, 1988; Larsson, Johansson, & Svenningsson, 1993). In legumes, RFOs accumulate during seed development and raffinose and stachyose are formed de novo during seed maturation (Frías, Díaz-Pollán, Hedley, & Vidal-Valverde, 1996a; Lowell & Kuo, 1989) and function as carbon reserves for use during germination (Dey, 1990; Frías et al., 1996b). α-Galactosides are soluble low-molecular weight oligosaccharides, such as raffinose (trisaccharide), stachyose, verbascose and other oligosaccharides formed by α-(1 → 6)-galactosides linked to C-6 of the glucose moiety of sucrose (Dey, 1985).

The right balance of intestinal bacterial flora is important for human health. In particular, the growth of *Bifidobacterium*, to dominate pathogenic organisms and thus invigorate human health, is facilitated by certain oligosaccharides (Alles et al., 1999; Salminen et al., 1998; Yanahira et al., 1995, 1997). Furthermore, it is known that diseases and ageing cause decay or a significant decrease of intestinal *Bifidobacteria*. Based on these facts, a lively interest in food additives that enhance human health has arisen. Some reports deal with the presence of specific oligosaccharides in human or animal nutrition that improve health by encouraging the growth of *Bifidobacteria* and so positively affect intestinal cells and the immune system

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(Fransen et al., 1998; Hamilton-Miller, 2000; Roberfroid, 1998).

Certain oligosaccharides, such as galacto-, isomalto-, and xylo-oligosaccharides, show favourable characteristics (Alles et al., 1999; Tomomatsu, 1994). Especially important is the role of indigestible α-(1–6) linked galactosyl-structures. These characteristics allow pure RFOs to be considered as prebiotics. Prebiotics are defined as non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and or activity of one or a limited number of bacteria in the colon (Fransen et al., 1998). RFOs promote the growth of *Bifidobacteria* population (Gibson & Roberfroid, 1995) and, consequently, there is a great deal of interest in using prebiotic oligosaccharides as ingredients in functional foods in order to manipulate the composition of colonic microflora, contributing to human health in many ways.

These low-molecular weight oligosaccharides are present in different seeds, but lupins are legumes with the highest α-galactoside contents (7–15%) (Frıas et al., 1996b; Muzquiz, Burbano, Pedrosa, Folkman, & Gulewicz, 1999; Ruiz-López et al., 2000; Van Kempen & Hughes, 1994) and sweet lupins can be a good source of RFOs. In this paper, we report a screening of RFOs and sucrose in 13 Spanish cultivars of lupin with the aim of selecting species with the highest amounts of RFOs. These galacto-oligosaccharides can easily be extracted and purified from lupin seeds to use them as functional food ingredients (prebiotics) (Gulewicz et al., 2000).

2. Materials and methods

2.1. Samples

The lupin seed samples used in this study were 13 cultivars obtained from the Agrarian Research and Technology Development Service from the Agriculture and Commerce Council of the Junta de Extremadura (Spain). Seeds were cleaned and stored in polyethylene containers in refrigeration at 4 °C until used.

2.2. Determination of soluble carbohydrate content

Sucrose and α-galactoside contents were determined by high performance liquid chromatography (HPLC), following the procedure described by Granito et al. (2002). The HPLC chromatograph (Waters Associates, Milford, CT) consisted of a Waters model 510 pump, a Rheodyne model 7000 sample injector, and a reflection type differential refractometer detector model R410 (Waters). The HPLC system was controlled by a PC with Maxima software (Waters). Chromatography was performed on a precolumn (0.32 cm i.d. × 4.0 cm) packed with C18 Porasil B and μ-Bondapak carbohydrate column (0.39 i.d. × 30 cm) (Waters). Acetonitrile: distilled water (75:25 v/v, HPLC grade) was used as the mobile phase at a flow rate of 2.0 ml/min. Solvents were filtered through a Millipore FH (0.45 μm) membrane and degassed under helium. Injection volumes were 100 μl.

Different amounts of sucrose, raffinose and stachyose standards (Merck, Germany) and samples, were dissolved in distilled water. Acetonitrile (HPLC-grade, ACROS-ORGANIC, Belgium) was added to each solution to obtain a composition similar to that of the mobile phase (75:25 v/v). Both lupin RFOs and standard solutions were filtered through a Millipore FH (0.45 μm) (Bedford, MA) membrane before injection. Quantification of each sugar was performed by comparing the peak areas with those of the standard solutions. Commercial verbascose standard was not available; for this reason, verbascose was quantified using stachyose as standard, based on previous works (Frıas, Hedley, Price, Fenwick, & Vidal-Valverde, 1994). Calibration curves were plotted for each sugar and adjusted by using the method of least squares. The regression coefficients of the curves for sucrose, raffinose and stachyose were always greater than 0.990.

3. Results

The sucrose content and oligosaccharide composition in seeds of six cultivars of *Lupinus albus*, three cultivars of *Lupinus luteus* and four cultivars of *Lupinus angustifolius* are shown in Table 1. Considerable variation in sucrose content was detected between species: *L. angustifolius* presented a range from 2.9% to 5.0%, *L. albus* from 2.2% to 3.1% and *L. luteus* from 1.0% to 1.4% (Table 1). Substantial differences in α-galactosides contents were also detected between species. Raffinose and stachyose were found in all lupin cultivars, but verbascose could not be detected in four cultivars of *L. albus.* The raffinose content ranged from 0.63% to 1.24% in the cultivars of *L. angustifolius*. from 0.54% to 0.64% in those of *L. luteus* and from 0.33% to 0.62% in the cultivars of *L. albus* studied (Table 1). Stachyose was always the main sugar present in lupin seeds. *L. luteus* and *L. albus* cultivars present high levels of stachyose (6.13–8.61% and 4.98–7.26%, respectively) while the *L. angustifolius* cultivars ranged from 3.62% to 5.19% (Table 1). The highest variation level for individual α-galactosides was found for verbascose, which was not detected in four lupin cultivars of *L. albus*. while *L. luteus* and *L. angustifolius* cultivars had 2.8–3.5% and 0.8–2.5%, respectively (Table 1). Total α-galactoside content in the lupin studied ranged from 5.30–12.3% (Table 1) and a wide variation between species was observed. *L. luteus* showed a remarkably high content of total α-galactosides (9.46–12.3%) which was about twice that of other lupin cultivars (Table 1).

Table 1
Soluble carbohydrate contents of 13 Spanish lupin cultivars[a]

Lupin cultivar	Sucrose (% d.m.)	Raffinose (% d.m.)	Stachyose (% d.m.)	Verbascose (% d.m.)	Total RFOs[b] (% d.m.)
L. albus cv. multolupa	2.58 ± 0.06	0.62 ± 0.03	5.74 ± 0.06	0.19 ± 0.10	7.56 ± 0.10
L. albus cv. marta	3.09 ± 0.08	0.33 ± 0.02	7.24 ± 0.11	0.94 ± 0.01	8.51 ± 0.13
L. albus LO-3844	2.16 ± 0.20	0.44 ± 0.03	7.26 ± 0.31	ND	7.71 ± 0.33
L. albus LO-3846	3.13 ± 0.06	0.54 ± 0.02	6.85 ± 0.03	ND	7.39 ± 0.03
L. albus LO-3848	2.41 ± 0.19	0.36 ± 0.02	5.71 ± 0.57	ND	6.07 ± 0.55
L. albus LO-3855	2.84 ± 0.09	0.48 ± 0.02	4.98 ± 0.22	ND	5.46 ± 0.24
L. luteus LO-4486	1.38 ± 0.13	0.56 ± 0.11	7.01 ± 1.22	3.54 ± 0.37	11.1 ± 1.69
L. luteus LO-4492	1.21 ± 0.04	0.64 ± 0.04	8.61 ± 0.20	3.04 ± 0.02	12.3 ± 0.20
L. luteus LO-4500	1.01 ± 0.09	0.54 ± 0.03	6.13 ± 0.31	2.79 ± 0.14	9.46 ± 0.41
L. angustifolius LO-4817	5.05 ± 0.22	1.24 ± 0.09	5.11 ± 0.36	2.48 ± 0.07	8.82 ± 0.43
L. angustifolius LO-4820	2.55 ± 0.24	0.89 ± 0.07	3.62 ± 0.06	0.79 ± 0.03	5.30 ± 0.12
L. angustifolius LO-4822	2.91 ± 0.19	1.15 ± 0.07	5.19 ± 0.14	1.36 ± 0.01	7.70 ± 0.11
L. angustifolius cv. zapaton	3.16 ± 0.17	0.63 ± 0.04	4.52 ± 0.18	1.39 ± 0.13	6.54 ± 0.34

[a] Mean value ± SD of four determinations.
[b] RFOs = Raffinose family oligosaccharides.

Stepwise discriminant analysis was applied to the data in order to differentiate lupin samples by considering the different varieties, using those compounds that were significantly different ($P \leqslant 0.05$) for each quantitative variable. Table 2 shows the means and the SDs of quantitative variables selected by the stepwise discriminant analysis, taking account of lupin varieties. The results of the application of the least significant difference (LSD), to compare the means for each group, are also included in this Table. The *L. albus* seeds were characterised by the lowest verbascose content, *L. luteus* seeds by the highest stachyose and verbascose and the lowest sucrose contents, and *L. angustifolius* seeds by the highest sucrose and the lowest stachyose contents.

Fig. 1 shows the lupin samples on the plane defined by the first two canonical variables for the lupin varieties. The lupin varieties differentiated 100% of the total correct classification (100% for those of *L. albus*, 100% for those of *L. luteus* and 100% for those of *L. angustifolius*) by the following variables: sucrose, raffinose, stachyose and verbascose. *L. albus*, *L. luteus* and *L. angustifolius* seeds were differentiated very well between them.

The multiple correlation carried out with the data showed that lupins with the highest level of sucrose also had the highest level of raffinose ($r = 0.49$) and the lowest content of stachyose ($r = -0.44$) ($P \leqslant 0.05$). The varieties with the highest content of raffinose had the lowest content of stachyose ($r = -0.42$) ($P \leqslant 0.05$).

4. Discussion

Trugo, Almeida, and Gross (1988) reported that there was a considerable variation in composition between different species, superimposed on environmental factors that may affect the carbohydrate composition of the grain. Sucrose and verbascose contents were genetically influenced whereas raffinose and also stachyose were dependent on environment (Trugo et al., 1988). A shorter vegetation period promotes a significant increase in stachyose and verbascose, and a decrease in the sucrose content. This effect is seemed to be more apparent in yellow lupin (*L. luteus*). Furthermore, Gorecki, Brenac, Clapham, Willcott, and Obedorf (1996) described the effect of maturation temperature on the composition of soluble carbohydrates in yellow lupin seeds. In these species, seed matured at 18 °C had more than twice the amount of stachyose and verbascose compared with seeds matured at 25 °C, such as *L. albus*.

Variations in the contents of soluble carbohydrates between legumes species are quite well established (Larsson et al., 1993; Vidal-Valverde, Frıas, & Valverde, 1993). The individual content of each α-galactoside seems to depend on the type of legume and also on the genotype. There are many works in the literature (Burbano, Muzquiz, Ayet, Cuadrado, & Pedrosa, 1999; Gulewicz et al., 2000; Jones, Barber, Arthur, & Hedley, 1999) which report a large variation of α-galactosides in different lines of lentils, peas and beans, both for the total RFOs and for the levels of individual

Table 2
Mean value ± SD of discriminant variables of lupin varieties

Lupin varieties	*n*	Sucrose	Raffinose	Stachyose	Verbascose
L. albus	18	2.70 ± 0.38[b]	0.46 ± 0.11[a]	6.30 ± 0.92[b]	0.36 ± 0.52[a]
L. luteus	8	1.17 ± 0.16[a]	0.59 ± 0.08[a]	7.36 ± 1.30[c]	3.09 ± 0.40[c]
L. angustifolius	12	3.42 ± 1.02[c]	0.98 ± 0.25[b]	4.61 ± 0.68[a]	1.51 ± 0.64[b]

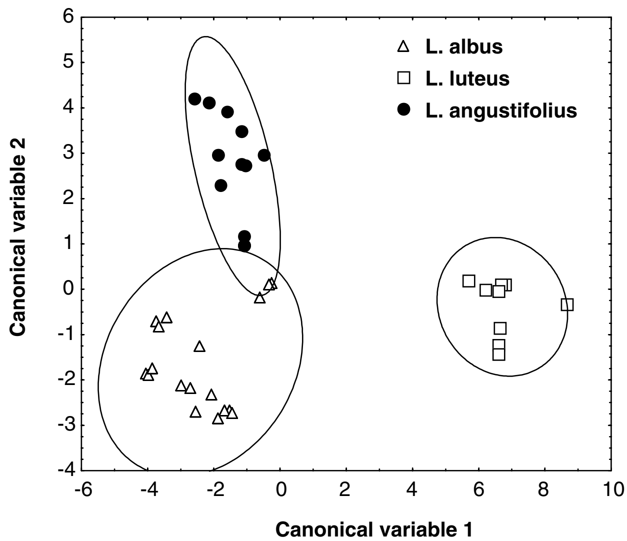

Fig. 1. Plot of lupin varieties in the plane defined by the first two canonical variables and population canonical ellipses for 95% confidence.

members. The main α-galactoside was stachyose, while variation was observed mainly for verbascose, the content of which dropped, in several cases to undetectable amounts. It is likely that is due to a block in the pathway at this point (Frías et al., 1999). Those cultivars which lacked verbascose had an increased level of stachyose, the previous homologue in the pathway.

Frías et al. (1996b) observed a sucrose increase at the early developmental stage which decreased later at maturation in lupin, pea and faba bean and Lowell and Kuo (1989) found the same in soybean. However, α-galactosides accumulated later in development: initially raffinose and then stachyose. In this way, it is observed that the formation of α-galactosides is accompanied by the disappearance of sucrose, which is a substrate for formation of these oligosaccharides. Moreover, there is a hypothesis, which explains correlation between high content of raffinose and low content of stachyose. Raffinose synthesis occurs early during seed development and then stops. Reserves of raffinose are converted to stachyose and verbascose later on during development (Obendorf, 1997). It can be concluded that *L. luteus* seeds were characterised by high stachyose, verbascose and total α-galactoside contents can be a good source of RFOs for use as prebiotics in functional foods.

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