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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).