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Functional lupin seeds (*Lupinus albus* L. and *Lupinus luteus* L.) after extraction of α-galactosides

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Abstract

Functional lupin seeds from two different cultivars of white (*Lupinus albus* L.) and yellow lupin (*Lupinus luteus* L.) each, were obtained by extraction of α-galactosides. The effect of extraction of α-galactosides from lupin seeds on different nutritional parameters (protein, fat, ash, dietary fibre, starch, sucrose, and vitamins B_1, B_2, E and C) and antinutritional factors (α-galactosides, trypsin inhibitor activity and inositol phosphates) were studied. In lupin seeds, α-galactosides were effectively removed and processed seeds contained very low amounts of flatulence causing factors (~0.5–1%). Protein, fat and starch contents showed high retention in processed seeds (up to ~130%). Sucrose and soluble dietary fibre, however, decreased significantly as a result of processing and retentions ranged from 10% to 60%, depending on the variety studied. Vitamins B_1, B_2, E and C were also reduced. Trypsin inhibitor activity was detected only in yellow lupin cultivars and inositol phosphate content was modified slightly after extraction. In summary, the functional lupin seeds, with low contents of α-galactosides, are a product of nutritional importance due to their high protein content, dietary fibre and fat contents as well as acceptable levels of thiamin, riboflavin and vitamin E. They can be incorporated as a proteic source, not only in animal feeding but also in a wide range of foods.
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1. Introduction

Due to animal protein sources often containing large amounts of saturated fat and cholesterol, most health organisations recommend the frequent consumption of vegetable protein, since it is known that it may reduce serum cholesterol levels, the risk of coronary heart diseases and diabetes. In consequence, the demand for healthy foods is increasing rapidly in developed countries and pulses have encountered a revival of interest among consumers.

Grain legumes are the main source of vegetable protein, among which lupin is known to have seeds with the highest protein content. In contrast to soybean, it can be cultivated in regions with mild climates, located in the Mediterranean, South America, Australia and New Zealand. The major cultivated species are *Lupinus albus* L. (white lupin), *Lupinus angustifolius* L. (blue lupin), *Lupinus luteus* L. (yellow lupin) and *Lupinus mutabilis* L. (pearl lupin). The first three lupin species belong to Mediterranean area while *L. mutabilis* belong to South America (Allen, 1998; Mülayim, Tamkoç, & Babaoglu, 2002). These species are known as sweet lupins due to their low levels (0.003%) of bitter-tasting and potentially toxic alkaloids (Petterson, 1998; Wäsche, Müller, & Knauf, 2001) and, therefore, there is no risk of toxicity for animals and humans.

Lupin seeds are a rich source of non-starch polysaccharides (30–40%), oil (5–15%) and protein (Faluyi et al., 2000; Huyghe, 1997; Petterson & Mackintosh,

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1994), at the same levels or sometimes above that of soybean, ranging from 30% to 40% in various species according to genotype and location (Erbas, Certel, & Uslu, 2005). Lupins contain a specific protein fraction, conglutin γ, which has the exceptional characteristic of being a sulphur-rich protein which contains amino acids that are scarce in other grain legumes.

It has been reported that lupin has lower levels of undesirable constituents, such as phytic acid and saponins, than soybean meal (Petterson & Fairbrother, 1996). In addition, lectins and protease inhibitors, that can reduce protein digestibility, are found at lower levels in lupin than in many other legumes (Petterson, Sipsas, & Mackintosh, 1997).

Due to its nutritional composition and satisfactory functional properties, lupin flour can be used in the production of fermented foods. In addition, there is an increased use of plant-derived ingredients as sources of vegetable protein in the formulation of food products such as dairy and meat analogues. They can be added to pasta, crisps, bread, biscuits and cakes and used for fine bakery and confectionery. The addition of up to 10% lupin flour improves water-binding, texture, shelf-life, aroma and nutritive value. Therefore, lupin has attracted interest, worldwide, as a potential high protein food ingredient suitable for human consumption (Fudiyansyah, Petterson, Bell, & Fairbrother, 1995; Johnson & Gray, 1993; Petterson & Crosbie, 1990).

However, there is a problem which has been considered to be the single most important factor that deters people from eating this nutritious food (Price, Lewis, Wyatt, & Fenwick, 1988). Lupins contain flatulence-causing factors, known as α-galactosides or the raffinose family of oligosaccharides (RFOs), that range from 7% to 15% of raw seeds (Glencross, 2001; Martínez-Villaluenga, Frias & Vidal-Valverde, 2005). Their consumption is associated with the production of flatulence because oligosaccharides are not hydrolysed in the small intestine, and they are fermented in the lower intestine (Cristofaro, Mottli, & Whurmann, 1974; Price et al., 1988). A particular food could be made more "functional" by increasing or adding a potential heath-promoting entity or, alternatively, reducing the concentration of known adverse components. Many investigations have been carried out to develop treatments in order to reduce or remove the content of α-galactosides and to enhance the nutritional quality of lupins. Some of these treatments applied to the seed are fermentation, germination, enzyme addition, dehusking, soaking and cooking (Doblado, Frias, Muñoz, & Vidal-Valverde, 2003; Khokhar, Frias, & Price, 1996; Vidal-Valverde et al., 1993a, Vidal-Valverde, Frías, & Valverde, 1993b). However, Gulewicz et al. (2000) developed a simple procedure for extracting α-galactosides from lentil and pea seeds which provide low α-galactoside-lupin seeds. The advantage of this procedure is that is not seed-destructive and the processed lupin seeds obtained can be incorporated in the design of novel proteic products. In addition, this procedure allows α-galactosides extracts to be obtained, which can be purified (Martínez-Villaluenga, Frías, Gulewicz, & Vidal-Valverde, 2004) and used as prebiotic ingredients (Villaluenga, Wardenska, Pilarski, Bernarczyk, & Gulewicz, 2004; Martínez-Villaluenga, Frías, Vidal-Valverde, & Gómez, 2005).

The object of this research was to evaluate the effect of α-galactoside extraction on different nutritive parameters (protein, fat, ash, dietary fibre, soluble carbohydrates, starch, vitamins B_1, B_2, E and C) and antinutritional factors (α-galactosides, inositol phosphates and trypsin inhibitor activity) of different varieties of white (*Lupinus albus* L.) and yellow lupin (*L. luteus* L.).

2. Materials and methods

2.1. Materials

2.1.1. Raw materials

Sweet lupin seeds from two varieties of *L. albus* and two varieties of *L. luteus* were obtained from the Agricultural Research and Technology Development Service of the Agriculture and Commerce Council of the Junta de Extremadura (Spain). Seeds were cleaned and stored in polyethylene containers at 4 °C until used.

2.1.2. Extraction of α-galactosides

Lupin seeds were submitted to several extractions according to a procedure described by Gulewicz et al. (2000). First of all, 100 g of legume seeds allowed to imbibe 200 ml of distilled water. The imbibing of seeds was carried out at 4 °C overnight. The imbibed seeds were then extracted with 200 ml of 50% ethanol (v/v) at 40 °C overnight. After extraction, the supernatant was decanted. The seeds were reextracted with fresh alcohol solutions under the same conditions. The supernatants from two cycles of extraction were removed from the seeds and used for purification of α-galactosides. Extracted seeds were freeze-dried for analysis.

2.2. Methods

2.2.1. Determination of water, protein, fat and ash contents

These analyses were carried out according to AOAC (1990).

2.2.2. Determination of sucrose content

Sucrose content was determined by HPLC, following the procedure described by Granito et al. (2002).

2.2.3. Determination of dietary fibre

Determinations of total dietary fibre, soluble and insoluble fractions were carried out according to Lee and Prosky (1992).

2.2.4. Determination of starch content

Total and available starch levels of samples were determined as in Doblado et al. (2003) by a procedure based on total enzyme digestion of starch to glucose for 3 h and 30 min, respectively.

2.2.5. Determination of vitamins

Determination of thiamin (vitamin B_1) and riboflavin (vitamin B_2) was carried out with a single extraction procedure according to Vidal-Valverde, Prodanov, and Sierra (1997) and these vitamins were quantified by high-performance liquid chromatography (HPLC) according to Prodanov, Sierra, and Vidal-Valverde (1997). The vitamin C was determined by capillary electrophoresis according to Thompson and Trenerry (1995). Determination of tocopherols by HPLC was carried out as described in Frías, Miranda, Doblado, and Vidal-Valverde (2004).

2.2.6. Determination of α-galactosides

α-Galactoside content was determined by HPLC, following the procedure described by Granito et al. (2002).

2.2.7. Determination of inositol phosphates

Inositol phosphates (hexa-[IP_6], penta-[IP_5], tetra-[IP_4] and tri-inositol [IP_3] phosphates) were extracted according to Kozlowska, Honke, Sadowska, Frías, and Vidal-Valverde (1996), while their quantification was carried out by HPLC according to Lehrfeld (1994).

2.2.8. Determination of trypsin inhibitor activity (TIA)

TIA was determined as in Vidal-Valverde et al. (1993a, 1993b).

2.3. Statistical analysis

All data were expressed as the means and standard deviations of quadruplicate determinations and were subjected to multifactor analysis of variance with the use of the least significance difference test with the Statgrafic 5.0 Program (Statistical Graphics Corporation, Rockville, MD).

3. Results and discussion

The nutritional compositions of different varieties of *L. albus* and *L. luteus* seeds are shown in Tables 1 and 2. Similar results for protein, fat and ash contents of raw lupin seeds have been reported in previous works (Derbas, Doxatakis, Hadjisavva-Zinoviada, & Triantafillakos, 1999; Erbas et al., 2005). The protein content of lupins tested (ranging from 30% to 40%) closely resembled that of soybean. Lupin generally contains about twice the protein found in those legumes normally consumed by humans. There was a variation in protein content between species and cultivars as a result of the

Table 1
Effect of α-galactosides extraction on nutritional value of white lupin (*Lupinus albus*) seeds

Nutritional components	*L. albus* var. Multolupa		*L. albus* var. Marta	
	Raw seeds	Processed seeds	Raw seeds	Processed seeds
Protein (g/100 g d.m.)	30.6 ± 0.26^a	39.0 ± 0.36^b	37.4 ± 3.12^a	45.3 ± 0.74^b
Fat (g/100 g d.m.)	14.64 ± 1.11^a	15.98 ± 0.00^a	11.34 ± 0.73^a	15.78 ± 0.80^b
Ash (g/100 g d.m.)	3.65 ± 0.29^b	2.12 ± 0.10^a	3.79 ± 0.06^b	2.21 ± 0.11^a
Soluble carbohydrates (g/100 g d.m.)				
Sucrose	2.58 ± 0.06^b	1.32 ± 0.09^a	3.09 ± 0.08^b	0.20 ± 0.01^a
Dietary fibre (g/100 g d.m.)				
Soluble	5.21 ± 0.18^b	2.53 ± 0.08^a	3.64 ± 0.12^b	2.65 ± 0.01^a
Insoluble	34.22 ± 0.08^a	33.85 ± 0.09^a	30.80 ± 0.11^b	31.24 ± 0.19^b
Total fibre	39.42 ± 0.26^b	36.38 ± 0.17^a	34.44 ± 0.23^b	33.89 ± 0.18^a
Starch (g/100 g d.m.)				
Total	3.27 ± 0.23^a	3.85 ± 0.19^b	2.81 ± 0.08^a	3.85 ± 0.20^b
Available	1.78 ± 0.11^a	2.21 ± 0.04^b	1.84 ± 0.13^a	2.33 ± 0.24^b
Vitamins (mg/100 g d.m.)				
α-Tocopherol	0.19 ± 0.01^b	0.16 ± 0.01^a	0.47 ± 0.02^b	0.12 ± 0.02^a
γ-Tocopherol	20.1 ± 0.86^b	7.87 ± 0.96^a	$51.6 \pm 0.5b^d$	9.26 ± 0.50^a
δ-Tocopherol	0.25 ± 0.02^b	0.18 ± 0.01^a	0.41 ± 0.02^b	0.19 ± 0.01^a
Vitamin E activity	2.21 ± 0.11^b	0.95 ± 0.10^a	5.64 ± 0.06^b	1.25 ± 0.08^a
Thiamin	0.36 ± 0.01^b	0.15 ± 0.01^a	0.34 ± 0.01^b	0.27 ± 0.02^a
Riboflavin	0.61 ± 0.04^b	0.30 ± 0.01^a	0.65 ± 0.05^b	0.21 ± 0.00^a
Vitamin C	6.48 ± 0.09^b	ND[a]	ND	ND

Mean values ± standard deviation ($n = 4$). Rows with common superscripts are not significantly different ($P \leqslant 0.05$) for each lupin variety studied.

Table 2
Effect of α-galactosides extraction on nutritional value of yellow lupin (*Lupinus luteus*) seeds

Nutritional components	*L. luteus* var. 4486		*L. luteus* var. 4492	
	Raw seeds	Processed seeds	Raw seeds	Processed seeds
Protein (g/100 g d.m.)	37.9 ± 2.44^{a}	46.2 ± 0.82^{b}	36.8 ± 0.77^{a}	44.4 ± 2.29^{b}
Fat (g/100 g d.m.)	8.79 ± 0.42^{a}	12.09 ± 0.20^{b}	8.54 ± 0.02^{a}	13.40 ± 0.03^{b}
Ash (g/100 g d.m.)	4.95 ± 0.12^{b}	3.82 ± 0.08^{a}	3.14 ± 0.07^{b}	2.14 ± 0.06^{a}
Soluble carbohydrates (g/100 g d.m.)				
Sucrose	1.38 ± 0.13^{b}	0.67 ± 0.03^{a}	1.21 ± 0.04^{b}	0.43 ± 0.05^{a}
Dietary fibre (g/100 g d.m.)				
Soluble	4.90 ± 0.03^{b}	2.91 ± 0.04^{a}	3.21 ± 0.05^{b}	0.79 ± 0.04^{a}
Insoluble	28.78 ± 0.01^{a}	28.73 ± 0.38^{a}	31.13 ± 0.28^{b}	31.27 ± 0.73^{b}
Total fibre	33.68 ± 0.04^{b}	31.64 ± 0.07^{a}	34.33 ± 0.34^{b}	32.06 ± 0.69^{a}
Starch (g/100 g d.m.)				
Total	4.53 ± 0.41^{a}	5.22 ± 0.29^{b}	4.00 ± 0.09^{a}	4.99 ± 0.10^{b}
Available	1.84 ± 0.13^{a}	2.68 ± 0.14^{b}	2.20 ± 0.24^{a}	3.00 ± 0.06^{b}
Vitamins (mg/100 g d.m.)				
α-Tocopherol	0.48 ± 0.01^{b}	0.30 ± 0.01^{a}	0.27 ± 0.02^{b}	0.10 ± 0.01^{a}
γ-Tocopherol	11.19 ± 0.63^{b}	5.72 ± 0.27^{a}	9.41 ± 0.16^{b}	5.65 ± 0.19^{a}
δ-Tocopherol	0.38 ± 0.01^{b}	0.18 ± 0.03^{a}	0.24 ± 0.01^{b}	0.12 ± 0.01^{a}
Vitamin E activity	1.61 ± 0.06^{b}	0.87 ± 0.02^{a}	1.22 ± 0.01^{b}	0.67 ± 0.02^{a}
Thiamin	1.49 ± 1.12^{b}	0.53 ± 0.08^{a}	1.16 ± 0.07^{b}	0.55 ± 0.06^{a}
Riboflavin	0.85 ± 0.04^{b}	0.20 ± 0.01^{a}	0.37 ± 0.02^{b}	0.16 ± 0.01^{a}
Vitamin C	2.56 ± 0.13^{b}	ND[a]	ND	ND

Mean values ± standard deviation ($n = 4$). Rows with common superscripts are not significantly different ($P \leqslant 0.05$) for each lupin variety studied.

characteristics of the growing conditions and soil type (Petterson et al., 1997), *L. luteus* cv. 4486 and *L. albus* var. Marta having the highest protein content of the lupins analyzed. Glencross (2001) reported that the fat content of lupins varied considerably between the different species and even cultivars. As a result, *L. luteus* generally had the lowest fat levels and *L. albus* the highest (Petterson, 2000; Petterson et al., 1997), in accordance with the results presented in Tables 1 and 2. Ash level was quite variable and it has been reported to be quite dependent on the soil type where the plant is grown (Petterson, 2000). This fact can explain differences in ash contents among lupin cultivars studied. Among yellow lupin cultivars, *L. luteus* cv. 4486 showed the highest ash content while for, *L. albus*, no important differences were found between cultivars.

The carbohydrate content of lupin seeds was quite different from that of most legumes (Van Barneveld, 1999). Lupin seeds are rich in soluble and insoluble fractions of non-starch polysaccharides as shown in Tables 1 and 2, where total fibre levels ranged from 34% to 40%, essentially double that of soybean (21.7%), peas (18%) and faba beans (19%) (Van Barneveld, 1999). Górecka, Lampart-Szczapa, Janitz, and Sokolowska (2000) have shown that fibre content of lupins varies considerably between the different species and even cultivars, which could explain results obtained in the present work in cultivars belonging to the *L. albus* specie. Lupins are typically low in starch, as observed in Tables 1 and 2, where available starch was no more than 2% in lupin species studied, results that agree with those reported by Mohamed and Rayas-Duarte (1995). Little variability appears to exist in the levels of available starch between cultivars while total starch values differ, depending on the species studied.

Lupin seed contained higher amounts of available soluble sugars than did wheat and others legumes, except for soybean (Favier, Ripert, Toque, & Feinberg, 1995). Considerable variation in sucrose content was detected only between species: *L. albus* from 2.6% to 3.1% and *L. luteus* from 1.2% to 1.4% (Tables 3 and 4, respectively) as was observed by other authors (Trugo, Almeida, & Gross, 1988; Vidal-Valverde et al., 1993a, 1993b).

The thiamin and riboflavin contents, in both raw lupin species, are in agreement with values reported by Petterson (2000). No differences between cultivars were found. Erbas et al. (2005) compared thiamin and riboflavin contents of lupin with other legumes (haricot bean, lentil and soybean) and wheat and they observed that thiamin content in lupins was lower than in other seeds analysed. Riboflavin lupin content was higher than wheat and haricot bean but it was lower than lentil and soybean. In the case of vitamin E, γ-tocopherol is the main isomer in the different cultivars studied. Differences were found in tocopherol levels between cultivars. *L. luteus* cv. 4486 presented higher tocopherol content and vitamin E activity than did *L. luteus* cv. 4492 and *L. albus* var. Marta and than *L. albus* var. Multolupa. Bramley et al. (2000) reported, that α-tocopherol was the most active homologue, followed by β-, γ- and δ-tocopherols. Vitamin C only was detected in raw seeds of *L. albus* var. Multolupa and *L. luteus* cv. 4486.

Table 3
Effect of α-galactosides extraction on antinutritional factors of white lupin (*Lupinus albus*) seeds

Antinutritional components	*L. albus* var. Multolupa		*L. albus* var. Marta	
	Raw seeds	Processed seeds	Raw seeds	Processed seeds
α-galactosides (g/100 g d.m.)				
Raffinose	0.62 ± 0.03^{b}	0.19 ± 0.01^{a}	0.33 ± 0.02^{b}	ND[a]
Stachyose	5.74 ± 0.03^{b}	0.34 ± 0.00^{a}	7.24 ± 0.11^{b}	0.60 ± 0.02^{a}
Verbascose	1.19 ± 0.10^{b}	ND[a]	0.94 ± 0.01^{b}	ND[a]
Total α-galactosides	7.56 ± 0.10^{b}	0.53 ± 0.01^{a}	8.51 ± 0.13^{b}	0.60 ± 0.02^{a}
Inositol phosphates				
IP6 (g/100 g d.m.)	0.25 ± 0.01^{a}	0.30 ± 0.01^{b}	0.33 ± 0.01^{a}	0.41 ± 0.03^{b}
IP5 (g/100 g d.m.)	ND	ND	ND	ND
IP4 (g/100 g d.m.)	ND	ND	ND	ND
IP3 (g/100 g d.m.)	ND	ND	ND	ND
Trypsin inhibitor (TIU/mg d.m.)	ND	ND	ND	ND

Mean values ± standard deviation ($n = 4$). Rows with common superscripts are not significantly different ($P \leqslant 0.05$) for each lupin variety studied.

Table 4
Effect of α-galactosides extraction on antinutritional factors of yellow lupin (*Lupinus luteus*) seeds

Antinutritional components	*L. luteus* var. 4486		*L. luteus* var. 4492	
	Raw seeds	Processed seeds	Raw seeds	Processed seeds
α-galactosides (g/100 g d.m.)				
Raffinose	0.56 ± 0.11^{b}	ND[a]	0.64 ± 0.04^{b}	0.06 ± 0.01^{a}
Stachyose	7.01 ± 1.22^{b}	0.46 ± 0.02^{a}	8.61 ± 0.20^{b}	0.90 ± 0.06^{a}
Verbascose	3.54 ± 0.37^{b}	ND[a]	3.04 ± 0.02^{b}	ND[a]
Total α-galactosides	11.11 ± 1.69^{b}	0.46 ± 0.02^{a}	12.29 ± 0.20^{b}	0.95 ± 0.06^{a}
Inositol phosphates				
IP6 (g/100 g d.m.)	0.61 ± 0.02^{a}	0.66 ± 0.02^{b}	0.66 ± 0.02^{a}	0.75 ± 0.02^{b}
IP5 (g/100 g d.m.)	ND	ND	0.18 ± 0.01^{b}	0.16 ± 0.00^{a}
IP4 (g/100 g d.m.)	ND	ND	ND	ND
IP3 (g/100 g d.m.)	ND	ND	ND	ND
Trypsin inhibitor (TIU/mg d.m.)	3.39 ± 0.32^{b}	4.01 ± 0.09^{b}	2.76 ± 0.10^{a}	2.29 ± 0.03^{a}

Mean values ± standard deviation ($n = 4$). Rows with common superscripts are not significantly different ($P \leqslant 0.05$) for each lupin variety studied.

Moreover, antinutritional factor contents of *L. albus* and *L. luteus* are presented in Tables 3 and 4, respectively. In raw seeds, trypsin inhibitor activity was only found in yellow lupin where differences between cultivars were found and *L. luteus* cv. 4492 showed the lowest content. Levels of protease inhibitors found in lupins analysed are very low in comparison with other legume seeds, particularly soybean (White, Campbel, & McDowel, 2000).

Lupin seeds showed a large amount of α-galactosides, in agreement with values reported in the literature (Glencross, 2001; Muzquiz, Burbano, Pedrosa, Folkman, & Gulewicz, 1999; Ruiz-López et al., 2000). Substantial differences in α-galactoside contents were detected between species and even among cultivars belonging to the *L. albus* specie. Stachyose was always the main α-galactoside present in lupin seeds, followed by verbascose. Finally, raffinose content was the lowest (compared with stachyose and verbascose levels). *L. luteus* showed a remarkably high content of total α-galactosides (~12%) which was about 1.5 times higher than white lupin cultivars.

Among inositol phosphate IP_6 (phytic acid) was present in lupin seeds studied, and IP_5 only in *L. luteus* cv. 4492, while IP_4 and IP_3 were not detected in any lupin cultivar studied.

The effect of extraction of α-galactosides on nutritional composition of different cultivars of white lupin (*L. albus* L.) and yellow lupin (*L. luteus* L.) is presented in Tables 1 and 2, respectively. After extraction of α-galactosides, protein content increased significantly ($P \leqslant 0.05$), up to 45% in both *L. albus* and *L. luteus* species with the exception of *L. albus* var. Multolupa (39%). Similar results have been reported by other authors in two different varieties of *L. angustifolius* seeds (Torres, Frias, & Vidal-Valverde, 2005). Due to the high protein content, lupin flour from low α-galactosides seeds, could be used in the human diet and for animal feeding with no risk of suffering flatulence problems. Furthermore, it would be necessary to emphasize the technological advantages of using lupin protein rather than other proteins: (1) temperature of denaturation of these proteins is higher than animal protein, therefore they are technologically easier to handle (Chapleau & Lamballerie-

Anton, 2003); (2) lupin protein has better solubility than soybean protein and has a similar emulsification capacity. They may, therefore, be considered as potential substitutes of soybean and other proteins in food manufacture (King, Aguirre, & DePablo, 1985). The fat content was significantly higher ($P \leqslant 0.05$) in processed seeds, reaching 16% and 13% in white and yellow lupins, respectively, which favours lupin use in nutrition due to its high proportion of unsaturated fatty acids, such as oleic (44%), linoleic (35%) and linolenic acid (2%) (Erbas et al., 2005; Feldheim, 1998). These results differ from that reported in the literature (Torres et al., 2005) in which fat content was maintained after ethanol treatment of *L. angustifolius* seeds. However, in contrast to protein and fat contents, after extraction ash content showed a significantly large reduction ($P \leqslant 0.05$).

After the α-galactoside extraction procedure, a significant ($P \leqslant 0.05$) reduction in soluble fibre was observed while little or no change occurred in insoluble fibre. This fact is important, due to the role of insoluble fibre in human physiology, resulting from its functional properties: water-holding capacity, cation-exchange capacity, gel-forming properties and bile acid absorption (Górecka et al., 2000). The available and total starch contents were significantly ($P \leqslant 0.05$) higher in low α-galactosides seeds of white and yellow lupins. Sucrose content, however, showed a significant ($P \leqslant 0.05$) reduction (>50%) after α-galactoside extraction.

Vitamins suffered a significant ($P \leqslant 0.05$) decrease after α-galactoside extraction as a result of solubilisation and instability of vitamins under extraction conditions. Thiamin content was reduced from 50% to 65% in most lupin seeds studied with the exception of *L. albus* var. Marta where only a 20% reduction was found. This last result conflicts with results obtained by Torres et al. (2005) for two varieties of *L. angustifolius* seeds. Riboflavin reduction was above 50%, in agreement with Torres et al. (2005). However, thiamin content of extracted samples still satisfies 10–50% of the Daily Requirement of a human consuming 2000 kcal/day (1.1 and 1.5 mg/day for women and men, respectively), while the riboflavin content satisfies 9.4–17.6% of the Daily Requirement (1.2 and 1.7 mg/day for women and men, respectively), depending on the lupin variety. There was a considerable decrease in vitamin E activity in *L. albus* var. Multolupa and *L. albus* var. Marta (57% and 77%, respectively) while *L. luteus* showed a similar reduction of vitamin E activity between cultivars (~45%). These results accord with those reported by Torres et al. (2005) for *L. angustifolius* seeds. Despite losses due to the extraction process, the remaining vitamin E content satisfies 6.7–18.5% of the Daily Recommendations for adults consuming 2000 kcal/day (10 and 8 mg/day for women and men, respectively).

When the extraction with ethanol was applied to lupin seeds, α-galactoside contents were sharply reduced (~0.5–1.0%). Coon, Leske, Akavanicham, and Cheng (1990) reported a removal of around 70% of α-galactosides and Gulewicz et al. (2000) 83% and 87% of total soluble sugars from pea and lentil seeds, respectively. However, results of the present work show a removal of approximately 99% of these compounds. On the other hand, α-galactosides collected during the extraction procedure can be utilised as prebiotic compounds, being included in food products of high added value, since they contribute to human health in many ways (Gulewicz et al., 2002; Van Loo et al., 1999).

α-Galactoside extraction slightly increased phytic acid content (Tables 3 and 4) in contrast to results of Torres et al. (2005), who reported that phytic acid content did not change after the extraction procedure. Phytic acid is considered as an antinutritional factor because of its capacity to form complexes with minerals, lowering their bioavailability, and decreasing protein digestibility. However, recently its antioxidant capacity has been reported (Claxon et al., 1990), and its effect on inactivating biologically active substances which play and important role in the development of colon cancer. Also, it may have cholesterol-lowering abilities (Urbano et al., 2000; Zhou & Erman, 1995).

Fig. 1 shows the remaining percentage, expressed as percentage of retention of nutritional and antinutrional content after extraction of α-galactosides, in *L. albus* and *L. luteus* varieties, respectively. The α-galactoside extraction process caused higher retentions of protein, fat and starch contents, important from a nutritional point of view. Furthermore, the content of insoluble fibre was maintained after the extraction of α-galactosides (near 100% retention). However, ash, sucrose, soluble fibre, and vitamins decreased significantly ($P \leqslant 0.05$) since they are hydrosoluble compounds which could be eluted by water during α-galactoside extraction. In lupin seeds, α-galactosides were removed with effectiveness, and processed seeds contained very low amounts of flatulence-causing factors. The inositol phosphates underwent an increase and retention observed was approximately 120% for phytic acid.

The effects of α-galactosides extraction on nutritional value of lupin seeds were increments of protein, fat and starch due to a concentration effect related to the decrease of other components which were solubilized. However, conditions used during the extraction process also affected other soluble compounds (ash, sucrose, soluble fibre, water-soluble vitamins) as observed by other authors (Torres et al., 2005).

In conclusion, α-galactoside extraction produces functional *L. albus* and *L. luteus* seeds which are very nutritive lupin products with high protein, dietary fibre and fat contents as well as acceptable levels of thiamin, riboflavin and vitamin E. Their flour can be incorporated as a functional ingredient in a wide range of foods and feedstuffs with no risk of flatulence problems.

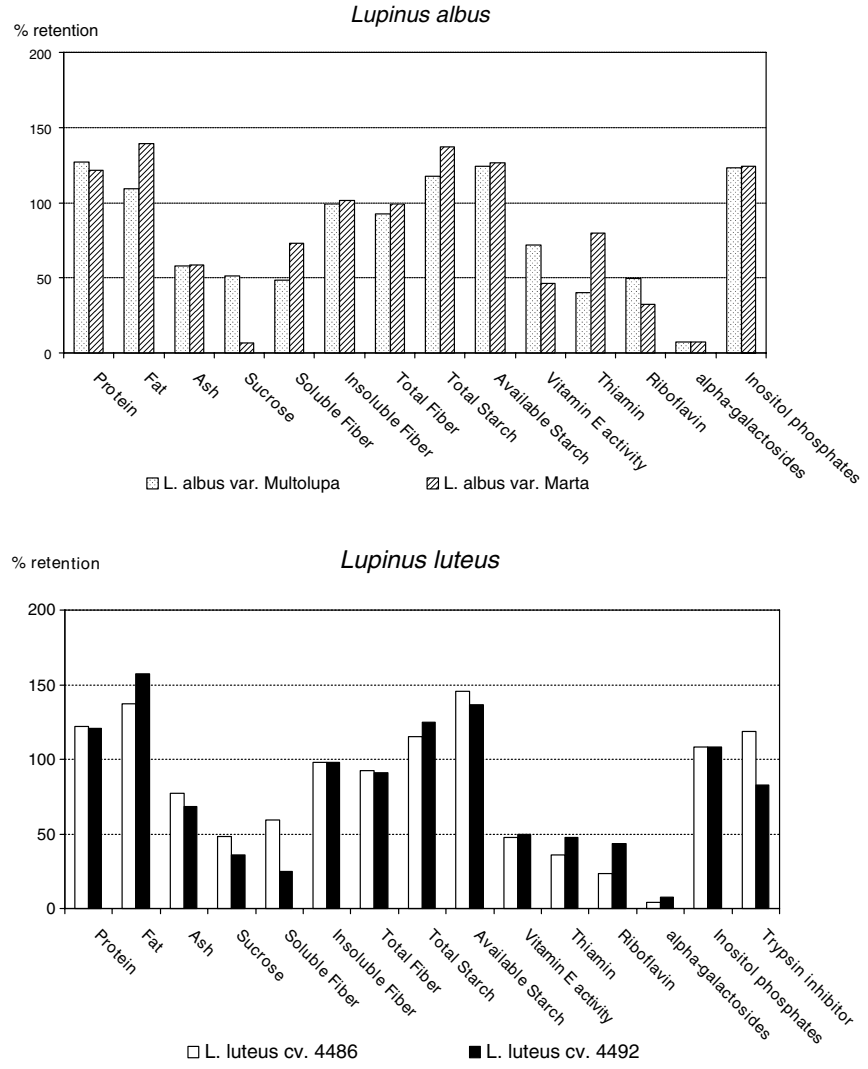

Fig. 1. Effects of processing on the retention of nutritional and antinutritional compounds in *Lupinus albus* and *Lupinus luteus*.

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