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The investigation of antinutritional factors in *Phaseolus vulgaris*. Environmental and varietal differences

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This study enables us to indicate that the oligosaccharide raffinose family, phytate, saponin and lectin contents of *P. vulgaris* are clearly influenced by both environmental and genetics factors. The results also indicate no relationship between the antinutritional factors analysed. From a nutritional point of view, these results would help investigators to select dry bean varieties with a high nutritive value (with a low content of α-galactosides, inositol phosphates, saponins and lectins) for human consumption and large-scale cultivation.
Keywords. *Phaseolus vulgaris*, antinutritional factors, genetic improvement, nutritional value, seed quality.

Recherche des facteurs antinutritionnels chez *Phaseolus vulgaris.* Variations environnementale et variétale. Cette étude démontre que les teneurs en oligosaccharides de la famille du raffinose, des phytates, des saponines et des lectines de *P. vulgaris* sont clairement influencées par des facteurs environnementaux et génétiques. Les résultats montrent également qu'il n'existe pas de relation entre les différents facteurs antinutritionnels analysés. D'un point de vue nutritionnel, ces données devraient aider les chercheurs dans la sélection de variétés de haricot sec à haute teneur nutritive (avec une faible teneur en α–galactosides, inositol phosphates, saponines et lectines) pour l'alimentation humaine et pour la culture à grande échelle.
Mots-clés. *Phaseolus vulgaris*, facteurs antinutritionnels, amélioration génétique, valeur nutritive, graines.

1. INTRODUCTION

A significant part of the human world population relies on legumes as a staple food for subsistence, particularly in combination with cereals. Legumes are often advocated in Western diets because of their beneficial nutritional effects and because they are a low cost source of protein (Borade *et al.*, 1984). However, they are under-used because of the content of antinutrient compounds, such as enzyme (trypsin, chymotrypsin, α-amylase) inhibitors, phytic acid, flatulence factors, saponins and toxic factors, lectins and the need for prolonged cooking (Lyimo *et al.*, 1992). Therefore, more information is needed about the potential nutritional implications of legume-based diets.

Indigestible substances include the flatulence-producing oligosaccharides, namely, raffinose, stachyose and verbascose which due to the absence of α-galactosidase in humans, are fermented anaerobically by micro-organisms to produce carbon dioxide, hydrogen and methane (Price *et al.*, 1988). Phytic acid binds trace elements and macro-elements such as zinc, calcium, magnesium and iron, in the gastrointestinal tract are making dietary minerals unavailable for absorption and utilisation by the body. It can also form complexes with proteins, proteases and amylases of the intestinal tract, thus inhibiting proteolysis (Tabekhia, Luh, 1980). Moreover, the phosphorus in phytate has been considered to be largely unavailable to the organism because of the limited capacity of monogastric species to hydrolyse phytate in the small intestine.

Other antinutritional factors are the saponins, which are composed of a steroidal, or triterpene aglycone linked to one or three saccharide chains of variable size and complexity via ester and ether linkages. Among the better-known biological effects of saponins is their capacity to cause lysis of erythrocytes (Khalil, El-Adawy, 1994) and to make the intestinal mucous membrane permeable (Johnson *et al.*, 1986). The main toxic components in *P. vulgaris* are lectins, sugar-binding proteins which bind and agglutinate red blood cells. The toxicity of lectins is characterized by growth inhibition in experimental animals and diarrhoea, nausea, bloating and vomiting in humans (Liener, 1982).

Saponins, α-galactosides, phytates and lectins are attracting considerable interest as a result of their

diverse properties, both deleterious and beneficial (Ali, Muzquiz, 1998).

Most of the research on Spanish dry beans has been related to varietal selection. The criteria for selection have always been resistance to disease or high yields, but never nutritive quality (Rodiño *et al.*, 1996). A study of the composition and nutritive quality of dry beans would therefore be of great interest, because the knowledge provided would also help to orient the work of investigators involved in varietal selection.

The aim of the present work is to determine the variation of some of those compounds, which are interesting from a nutritional point of view, for some selected varieties grown in Spain. The results obtained should permit us to select a number of varieties (of all variety-locality combinations assayed) to be used for large scale cultivation in Spain, and to verify the influence of variety and locality on antinutrients and the existence of relationships between some antinutritional factors.

2. EXPERIMENTAL

2.1. Materials

Ten varieties of dry beans from Castilla-León: 78/94 and 77/94 grown in Castilla-León and Bolita, Planchada, Riñón, Orbigo, Cardeno, Canela, Palmeña, Morada Larga grown in two Spanish areas: Castilla-León (V) and the Basque Country (PV); two varieties from Asturias (A): Andecha and Bonafema ; two varieties from the Basque Country (BC): Pinta Alavesa and Tolosana and five varieties from Valencia (Va): 1, 2, 3, 4 and 5 were used in this study. The varieties from Asturias, the Basque Country and Valencia have been cultivated in the same region they originated. All the selected varieties were considered to be inbred lines.

2.1.1. Environmental growth conditions. Castilla-León: 12.7 ºC annual mean temperature; 333.8 mm annual precipitation ; sandy soil; rate of N fertilization (21%): 200 kg·ha^{-1}.

The Basque Country: 12.1 ºC annual mean temperature; 595.4 mm annual precipitation; clayey soil; rate of N fertilization (18%): 500 kg·ha^{-1}.

In both areas common pesticides were used.

2.2. Methods

2.2.1. Raffinose oligosaccharides. Oligosaccharides were extracted from bean flour as described by Muzquiz *et al.* (1992). Bean flour (0.5 g) was homogenized in aqueous ethanol (80% v/v, 5 mL) for one minute at room temperature using an Ultraturrax homogenizer. The mixture was centrifuged for five minutes at 700 *g*, the supernatant decanted and the procedure repeated twice. The sample extract was purified using Dowex 50W × 8 (200–400 mesh) and QMA minicolumns using a vacuum system. The effluent was evaporated to dryness, redissolved in deionised water and determined by HPLC using a Spherisorb-5-NH2 column (250 × 4.6 mm id.) with acetonitrile:water 65:35 (v/v) (1 mL/min), as the mobile phase. A Beckman HPLC System Gold (USA) consisting of a pump, a refractive index detector and a Rheodyne injection valve (20 µL loop) and an electronic integrator was used.

2.2.2. Determination of inositol phosphates. The individual inositol phosphates (IP6–IP3) were extracted according to Burbano *et al.* (1995) with some modifications and determined by the Lehrfeld (1994) method. The sample (0.5 g) was extracted with 5 mL of 0.5 M HCl using shaking for 3 h. The extract (2.5 mL) was diluted with 25 mL of water and placed onto a SAX column (Varian). The column was washed with 2 mL of water, and then the inositol phosphates were eluted with 2 mL of 2 M HCl. The eluate was evaporated to dryness and the residue was dissolved in a buffer solution. The solution was centrifuged at 10,000 rpm for 6 min to remove any suspended material prior to injection into the HPLC. The column consisted of a macroporous polymer PRP–1 5 mm (150 × 4.1 mm, 5 mm) which was used at 45 °C. A Beckman System Gold HPLC equipment with a refractive index detector, an electronic integration system and a fixed loop (10 mL) injection valve (USA) was used.

2.2.3. Saponins. The extraction method was as described in Muzquiz *et al.* (1993). The qualitative analyses were conducted by thin layer chromatography (TLC) using two chromatographic systems:
– Reversed phase C18 bonded to silica gel (Whatman); methanol:water (3:2), and
– Silica gel 60 F_{254} (Merck); chloroform:methanol: water (65:35:10, lower layer). Both plates were sprayed with p-anisaldehyde:glacial acetic acid: concentrated sulphuric acid reagent (1:100:2).

The quantitative analyses were made by gas liquid chromatography of the corresponding aglycones (sapogenols), released as a result of acid hydrolysis (Ayet *et al.*, 1997). One microlitre of each sample was injected into a Perkin Elmer (Autosystem) gas chromatograph fitted with a SPB-1 capillary column (30 m × 0.25 mm id., 0.25 mm) and flame ionisation detector. The chromatographic conditions and calibration curve were as in Cuadrado *et al.* (1995).

2.2.4. Lectins. A competitive indirect ELISA assay for quantification of PHA (*P. vulgaris* lectin) was

performed as previously described (Hajós *et al.*, 1995) with some modifications. Plates coated overnight at 4 °C with 1mg/mL PHA (in 0.01M phosphate-buffered saline). Standard PHA diluted in PBS or kidney bean samples with unknown content of lectin were added, followed by rabbit anti-PHA IgG antibody (RRI, Aberdeen, UK, diluted 1:2000 with PBS). After incubation for 1 h, goat anti-rabbit IgG biotin conjugate (Sigma Chemical Co, St Louis, MO, USA) diluted with PBS (1:10000 v/v) was added. After washing, ExtrAvidin-peroxidase (Sigma Chemical Co, St Louis, MO, USA) diluted 1:500 was added. After incubation for 1 h a solution of OPD (o-phenylene-diamine)-H_2O_2 was added to each well and the reaction was stopped by adding 0.05 mL of 3M H_2SO_4 and the optical density measured at 492 nm.

2.2.5. Statistical analysis. The data were analysed using BMDP-7D (ANOVA) and correlation using BMDP-8D programs (WJ Dixon, BMDP Statistical Software Release, 1990) and the mean values compared using Duncan's multiple range test.

3. RESULTS AND DISCUSSION

3.1. Raffinose oligosaccharides

Flatulence factors (raffinose, stachyose and verbascose) and sucrose contents of 19 dry bean varieties proceeding from different growing Spanish areas are presented in **table 1**. Stachyose was the major α-galactoside contained in all the samples analysed, which also contained significant quantities of raffinose

Table 1. Sucrose, raffinose and oligosaccharide contents ($g \cdot kg^{-1}$) of dry bean varieties (mean ± SE, n = 4). a) Varieties from different Spanish areas; b) varieties from Castilla-León grown in the Basque Country — *Teneurs en sucrose, raffinose et oligosaccharides ($g \cdot kg^{-1}$) chez des variétés de haricot sec ($\bar{X}$ ± s, n = 4). a) Variétés provenant de diverses régions d'Espagne ; b) variétés originaires de Castilla-León cultivées au Pays Basque.*

Variety	Sucrose	Raffinose	Stachyose	Verbascose	Total galactosides
a) V78/94 (**1**)	27.5 ± 0.8	3.3 ± 0.1	25.5 ± 0.6	ND (**6**)	28.7 ± 0.7
V77/94	24.2 ± 0.1	3.1 ± 0.0	22.5 ± 0.2	ND	25.6 ± 0.2
VBolita	15.7 ± 0.2	2.6 ± 0.3	27.0 ± 0.3	1.3 ± 0.1	30.8 ± 0.4
VPlanchada	21.2 ± 0.1	2.9 ± 0.0	27.0 ± 0.2	0.4 ± 0.1	30.2 ± 0.3
VRiñón	17.5 ± 0.3	2.4 ± 0.1	26.7 ± 0.5	1.5 ± 0.1	30.6 ± 0.5
VOrbigo	15.4 ± 0.3	2.8 ± 0.2	22.7 ± 0.4	1.7 ± 0.1	27.2 ± 0.6
VCárdeno	21.1 ± 1.2	2.4 ± 0.3	28.1 ± 1.8	2.7 ± 0.5	33.2 ± 2.6
VCanela	26.1 ± 0.4	1.3 ± 0.2	27.6 ± 0.4	1.0 ± 0.1	30.0 ± 0.3
VPalmeña	14.1 ± 0.2	1.0 ± 0.0	29.3 ± 0.3	1.1 ± 0.1	31.4 ± 0.2
VMorada Larga	15.2 ± 0.1	2.7 ± 0.1	24.6 ± 0.1	2.3 ± 0.1	29.5 ± 0.2
BCPinta Alavesa (**2**)	24.9 ± 0.5	1.7 ± 0.1	24.4 ± 0.4	1.9 ± 0.2	28.0 ± 0.5
BCTolosana	21.2 ± 0.6	1.9 ± 0.1	20.9 ± 0.6	1.2 ± 0.1	24.0 ± 0.8
ABonafema (**3**)	18.5 ± 0.3	2.7 ± 0.1	28.8 ± 0.5	1.3 ± 0.1	32.8 ± 0.6
AAndecha	22.0 ± 0.2	2.5 ± 0.1	24.5 ± 0.2	1.9 ± 0.2	28.9 ± 0.4
Va 1 (**4**)	18.7 ± 0.7	3.6 ± 0.1	24.3 ± 0.1	1.3 ± 0.1	29.1 ± 0.2
Va 2	22.1 ± 0.5	4.3 ± 0.8	20.8 ± 0.5	0.8 ± 0.0	26.0 ± 1.3
Va 3	17.3 ± 0.0	5.6 ± 0.5	27.3 ± 0.2	1.8 ± 0.3	34.7 ± 0.0
Va 4	19.2 ± 0.5	5.2 ± 0.1	27.8 ± 0.1	1.4 ± 0.1	34.4 ± 0.1
Va 5	12.8 ± 0.2	4.4 ± 0.0	25.7 ± 0.1	1.9 ± 0.2	32.0 ± 0.1
b) PVBolita (**5**)	19.7 ± 0.6	2.7 ± 0.2	20.8 ± 0.1	ND	23.5 ± 0.2
PVPlanchada	22.4 ± 0.0	2.5 ± 0.2	19.1 ± 0.1	ND	21.6 ± 0.1
PVRiñón	23.2 ± 0.7	1.6 ± 0.0	23.9 ± 0.6	0.9 ± 0.0	25.2 ± 0.3
PVOrbigo	23.9 ± 0.4	2.4 ± 0.2	19.9 ± 0.3	0.9 ± 0.0	22.7 ± 0.9
PVCárdeno	25.2 ± 0.5	1.0 ± 0.1	21.5 ± 0.0	1.0 ± 0.3	23.6 ± 0.5
PVCanela	28.9 ± 0.1	1.0 ± 0.0	23.7 ± 0.1	1.0 ± 0.0	25.8 ± 0.1
PVPalmeña	21.4 ± 0.1	0.9 ± 0.0	24.2 ± 0.2	1.5 ± 0.1	26.6 ± 0.2
PVMorada Larga	22.2 ± 0.1	ND	18.3 ± 0.0	0.7 ± 0.0	18.6 ± 0.4

(**1**) V: originated from Castilla-León area. (**2**) BC: originated from the Basque Country. (**3**) A: originated from Asturias area. (**4**) Va: originated from Valencia area. (**5**) PV: originated from Castilla-León area and grown in the Basque Country. (**6**) ND: not detected.

and verbascose. The varieties ranged from 0.9 g·kg^{-1} (PVPalmeña) to 5.6 g·kg^{-1} (Va 3) for raffinose, from 18.3 g·kg^{-1} (PVMorada L.) to 29.3 g·kg^{-1} (VPalmeña) for stachyose and from 0.4 g·kg^{-1} (VPlanchada) to 2.7 g·kg^{-1} (VCárdeno) for verbascose. Similar concentrations have been reported for dry beans grown in Canada (Sosulski *et al.,* 1982) and in Burundi (Barampama, Simard, 1993). Results of variance analysis indicated that the total raffinose oligosaccharide concentrations of dry bean varieties were significantly different ($p < .001$) but were not influenced by locality ($p > .05$).

3.2. Inositol phosphates

Data on individual and total inositol phosphates of dry beans are summarised in **table 2**. The total inositol phosphate contents determined by ion-pair HPLC showed significant differences among the varieties and ranged from 2.9 g·kg^{-1} (Va 1) to 5.0 g·kg^{-1} (PVBolita). These results are lower than those previously reported for this food legume (Lolas, Markakis, 1975; Eeckhout, de Paepe, 1994). However, the legume seeds used for this study were obtained from different areas in Spain. The climatic conditions, and soil type in which seeds were grown and the variety all affect the inositol phosphate content (Burbano *et al.*, 1995). Moreover, some of the literature values were obtained using colorimetric methods (Lolas, Markakis, 1975) which have been demonstrated to be less accurate and precise than HPLC methodology (Burbano *et al.*, 1995; Xu *et al.*, 1992).

In the present study the application of an ion-pair HPLC method enables us to analyse in a greater detail

Table 2. Inositol phosphate contents (g·kg^{-1}) of dry bean (*P. vulgaris*) varieties (mean ± SE, n = 4). a) Varieties from different Spanish areas; b) varieties from Castilla-León grown in the Basque Country — *Teneurs en inositol phosphate (g·kg-1) chez des variétés de haricot sec (*P. vulgaris*) ($\bar{X}$ ± s, n = 4). a) Variétés provenant de diverses régions d'Espagne; b) variétés originaires de Castilla-León cultivées au Pays Basque.*

Variety	IP3	IP4	IP5	IP6	Total
a) V78/94 (**1**)	ND (**6**)	ND	0.39 ± 0.03	4.15 ± 0.15	4.54 ± 0.17
V77/94	ND	ND	0.39 ± 0.03	4.21 ± 0.19	4.60 ± 0.22
VBolita	ND	0.04 ± 0.00	0.38 ± 0.02	3.23 ± 0.13	3.63 ± 0.15
VPlanchada	0.04 ± 0.00	0.04 ± 0.00	0.53 ± 0.04	3.44 ± 0.24	4.00 ± 0.28
VRiñón	0.01 ± 0.00	0.01 ± 0.00	0.36 ± 0.03	4.42 ± 0.09	4.79 ± 0.09
VOrbigo	0.01 ± 0.00	ND	0.33 ± 0.02	3.85 ± 0.09	4.19 ± 0.11
VCárdeno	ND	0.13 ± 0.05	0.82 ± 0.24	2.52 ± 0.56	3.48 ± 0.30
VCanela	ND	ND	0.31 ± 0.01	3.30 ± 0.01	3.62 ± 0.02
VPalmeña	ND	ND	0.49 ± .05	4.46 ± 0.01	4.95 ± 0.04
VMorada Larga	ND	ND	0.37 ± 0.02	3.31 ± 0.14	3.69 ± 0.16
BCPinta Alavesa (**2**)	ND	0.08 ± 0.01	0.75 ± 0.05	3.45 ± 0.40	4.28 ± 0.45
BCTolosana	ND	0.08 ± 0.02	0.84 ± 0.02	3.50 ± 0.16	4.41 ± 0.17
ABonafema (**3**)	ND	0.10 ± 0.06	0.64 ± 0.02	2.79 ± 0.25	3.54 ± 0.33
AAndecha	ND	0.11 ± 0.07	0.47 ± 0.02	2.72 ± 0.20	3.30 ± 0.16
Va 1 (**4**)	ND	ND	0.22 ± 0.01	2.67 ± 0.05	2.89 ± 0.07
Va 2	ND	ND	0.33 ± 0.04	3.14 ± 0.24	3.47 ± 0.28
Va 3	ND	0.11 ± 0.03	0.60 ± 0.01	2.70 ± 0.01	3.40 ± 0.03
Va 4	ND	0.10 ± 0.00	0.59 ± 0.03	2.78 ± 0.09	3.47 ± 0.12
Va 5	ND	0.09 ± 0.01	0.52 ± 0.06	2.49 ± 0.33	3.10 ± 0.41
b) PVBolita (**5**)	0.01 ± 0.00	0.35 ± 0.27	0.77 ± 0.03	3.89 ± 0.07	5.01 ± 0.16
PVPlanchada	ND	0.10 ± 0.01	0.92 ± 0.02	3.77 ± 0.02	4.78 ± 0.01
PVRiñón	0.01 ± 0.00	0.06 ± 0.00	0.72 ± 0.04	3.94 ± 0.29	4.73 ± 0.33
PVOrbigo	ND	0.03 ± 0.02	0.56 ± 0.01	2.85 ± 0.07	3.44 ± 0.04
PVCárdeno	ND	0.05 ± 0.00	0.56 ± 0.06	3.26 ± 0.04	3.87 ± 0.09
PVCanela	0.01 ± 0.00	0.08 ± 0.01	0.80 ± 0.05	3.74 ± 0.12	4.63 ± 0.16
PVPalmeña	ND	0.07 ± 0.01	0.70 ± 0.00	3.58 ± 0.19	4.35 ± 0.19
PVMorada Larga	ND	0.07 ± 0.01	0.70 ± 0.00	3.63 ± 0.06	4.40 ± 0.07

(**1**) V: originated from Castilla-León area. (**2**) BC: originated from the Basque Country. (**3**) A: originated from Asturias area. (**4**) Va: originated from Valencia area. (**5**) PV: originated from Castilla-León area and grown in the Basque Country. (**6**) ND: not detected.

the composition of inositol phosphates of the dry bean varieties. The relative percentage values obtained indicate that most dry beans contain more than 80% of its inositol phosphates in the form of IP6. The relative proportion of IP5 ranges from 7.5% in the VRiñón variety to 24% in the VCárdeno variety both from Castilla-León area. The relative percentages of IP3 and IP4 are low and never higher than 4% except PVBolita. Only the highly phosphorilated inositol phosphates IP5 and IP6 have a negative effect on the bioavailability of minerals, the other hydrolytic products formed have a poor capacity to bind mineral, or the complexes formed are more (Sandberg *et al.*, 1989).

Our data clearly indicate the existence of a wide range of variation in inositol phosphates composition among the tested varieties of dry beans. This could be used to improve the quality of *P. vulgaris* by plant breeding.

3.3. Saponins

The native saponins were determined qualitatively by TLC using as standard soyasaponin I and the aglycones, soyasapogenols were quantified by capillary gas chromatography.

The TLC results revealed that the bean seeds contain soyasaponin I and V with a Rf of 0.22 and 0.27 respectively in the chromatographic system A, and 0.15 and 0.07 in the system B. Furthermore, other compounds with similar Rf to soyasaponin I appeared in system B. They could correspond to other monodesmoside saponins. It would be necessary to apply other techniques, such as fast atom bombardment-mass spectrometry, to identify these unknown compounds.

In a previous report, Ayet *et al.* (1996) established by FAB-MS that *P. vulgaris* contained a mixture of both soyasaponin I and soyasaponin V, soyasaponin V being the major saponin of *P. vulgaris*. Capillary gas chromatography of the bean samples revealed only one peak corresponding to soyasapogenol B, according to the retention time of this compound (22 min). Since acid hydrolysis released soyasapogenol B aglycone, which is common to both saponins I and V, the total saponin content for each sample was estimated as soyasapogenol B (**Table 3**). Analysis of variance indicated significant differences ($p < .001$) among varieties. The lowest soyasapogenol B mean value corresponds to the ABonafema variety (0.44 g·kg^{-1}) while the VBolita variety has the highest mean value (2.05 g·kg^{-1}).

Table 3. Soyasapogenol B (g·kg^{-1}) content in dry bean varieties (mean ± SE, n = 4) — *Teneurs en soyasapogénole B (g·kg^{-1}) chez des variétés de haricot sec ($\bar{X}$ ± s, n = 4).*

Variety	Soyasapogenol B
V78/94 (**1**)	1.93 ± 0.37
V77/94	1.79 ± 0.48
VBolita	2.05 ± 0.26
VPlanchada	1.18 ± 0.07
VRiñón	1.57 ± 0.28
VOrbigo	0.89 ± 0.36
VCárdeno	1.28 ± 0.20
VCanela	1.70 ± 0.39
VPalmeña	1.98 ± 0.54
VMorada Larga	1.61 ± 0.63
BCPinta Alavesa (**2**)	1.40 ± 0.50
BCTolosana	1.03 ± 0.22
ABonafema (**3**)	0.44 ± 0.20
AAndecha	0.92 ± 0.28

(**1**) V: originated from Castilla-León area. (**2**) BC: originated from the Basque Country. (**3**) A: originated from Asturias area.

3.4. Lectins

The major antinutritional toxic factor limiting the use of *P. vulgaris* is lectin (PHA). Although, in general, lectins are more resistant to heat-denaturation than other plant proteins, prolonged cooking can inactivate legume lectins. However, as heat-processing is expensive and potentially damaging, it is usually kept to a minimum even with legumes, particularly when the product is to be used in animal nutrition (Pusztai, Bardocz, 1996). **Table 4** shows the data obtained for the lectin content of the different bean varieties measured using ELISA methodology. The content of PHAranged from 1.89 g·kg^{-1} (VPlanchada) to 9.99 g·kg^{-1} (AAndecha). According to the results obtained, the lectin content of dry bean varieties were significantly different ($p < .001$).

3.5. Relationship between locality (Castilla-León / Basque Country) and variety

The concentrations of total α-galactosides, inositol phosphates and lectins, were determined in 16 "variety/locality" combinations of dry beans grown in Spain. The results are shown in **table 5**. In this experiment 8 of the 19 (with V78/94 and V77/94) varieties studied were grown in two areas (Castilla-León and the Basque Country) which have marked differences in environmental and growth conditions, as mentioned previously. Although both areas have similar annual mean temperatures, Castilla-León has more extreme maximum and minimum temperatures (32.1 °C and -0.2 °C) than the Basque Country (27.5 °C and 1.9 °C).

Results of variance analysis indicated that α-galactoside concentrations were influenced by locality ($p < .001$), variety ($p < .01$) and by the interaction of

Table 4. Lectin content (g·kg^{-1}) in dry bean varieties (mean ± SE, n = 4) — *Teneurs en lectines (g·kg^{-1}) chez des variétés de haricot sec ($\overline{X}$ ± s, n = 4)*.

Variety	PHA
V78/94 (**1**)	1.90 ± 0.1
V77/94	3.17 ± 0.4
VBolita	4.48 ± 0.0
VPlanchada	1.89 ± 0.3
VRiñón	5.33 ± 0.2
VOrbigo	4.52 ± 0.5
VCárdeno	7.69 ± 0.4
VCanela	4.88 ± 0.1
VPalmeña	4.88 ± 0.1
VMorada Larga	7.13 ± 0.4
BCPinta Alavesa (**2**)	5.50 ± 0.5
BCTolosana	6.49 ± 1.5
ABonafema (**3**)	7.77 ± 1.4
AAndecha	9.99 ± 0.3
Va 1 (**4**)	5.74 ± 0.5
Va 2	9.00 ± 0.4
Va 3	6.19 ± 0.4
Va 4	4.99 ± 0.6
Va 5	4.35 ± 0.1

(**1**)V:originated from Castilla-León area. (**2**)BC: originated from the Basque Country. (**3**) A: originated from Asturias area. (**4**) Va: originated from Valencia area.

Table 5. α-galactoside, inositol phosphate and PHA contents (g·kg^{-1}) in dry beans varieties grown in two Spanish localities (mean ± SE, n = 4) — *Teneurs en α–galactosides, inositol phosphate et PHA (g·kg^{-1}) chez des variétés de haricot sec cultivées dans deux localités d'Espagne ($\overline{X}$ ± s, n = 4)*.

Locality × variety combination	Total α-galactosides	Total inositol phosphates	PHA
VBolita (**1**)	30.8 ± 0.4	3.63 ± 0015	4.48 ± 0.2
PVBolita (**2**)	23.5 ± 0.2	5.01 ± 0.16	1.77 ± 0.2
VPlanchada	30.2 ± 0.3	4.00 ± 0.28	1.89 ± 0.3
PVPlanchada	21.6 ± 0.1	4.78 ± 0.01	1.28 ± 0.3
VRiñón	30.6 ± 0.5	4.79 ± 0.09	5.33 ± 0.2
PVRiñón	25.2 ± 0.3	4.73 ± 0.33	1.65 ± 0.1
VOrbigo	27.2 ± 0.6	4.19 ± 0.11	4.52 ± 0.5
PVOrbigo	22.7 ± 0.9	3.44 ± 0.04	5.09 ± 0.5
VCárdeno	33.2 ± 2.6	3.48 ± 0.30	7.69 ± 0.6
PVCárdeno	23.6 ± 0.5	3.87 ± 0.09	1.34 ± 0.1
VCanela	30.0 ± 0.3	3.87 ± 0.09	4.88 ± 0.1
PVCanela	25.8 ± 0.1	4.63 ± 0.16	5.13 ± 0.1
VPalmeña	31.4 ± 0.2	4.95 ± 0.04	5.32 ± 0.8
PVPalmeña	26.6 ± 0.2	4.35 ± 0.19	2.79 ± 0.1
VMorada L.	29.5 ± 0.2	3.69 ± 0.16	7.13 ± 0.3
PVMorada L.	18.6 ± 0.4	4.40 ± 0.07	6.52 ± 0.9

(**1**) V: grown in Castilla-León area. (**2**) PV: grown in the Basque Country.

variety and locality ($p < .05$); phytate concentrations were also influenced by locality, variety and by the interaction between both factors ($p < .01$). The seeds grown in the Basque Country showed significantly α-galactoside lower content than the same varieties grown in Castilla-León. On the other hand, highly significant differences by locality ($p < .01$), variety ($p < .001$) and the variety-locality interaction ($p < .001$) were also observed for lectin content. The seeds grown in the Basque Country had lower lectin content than those grown in Castilla-León, except for the PVOrbigo and PVCanela varieties. So, α-galactosides and lectin contents showed a similar trend with those varieties from the Basque Country having lower values than those from Castilla-León did. Pusztai *et al.* (1979) and Sosulski *et al.* (1982) also reported similar findings. These authors found significant differences in seed antinutritional factors (lectins, trypsin inhibitors and tannins) contents among dry beans varieties grown in the USA. Barampama and Simard (1993) found same trends in bean varieties grown in Burundi. Furthermore, numerous reports are available on the interactions between environmental and genetic factors on protein content and nutritive value of different legumes (Bresanni, Elias, 1980).

Barampama and Simard (1993) detected significant negative correlations between some nutrients (protein concentration-carbohydrate and protein-fat) but found no correlation between antinutritional factors and protein concentration. Neither did any relationship exist, according to Barampama and Simard (1993), between lectins, phytic acid or saponin contents and *in vitro* digestibility of protein. A significant negative correlation between tannin concentration and protein digestibility was, however, reported by Aw and Swanson (1985). Barampama and Simard (1993) observed a negative correlation between trypsin inhibitor and protein digestibility for the dry bean varieties analysed. In the present study no correlation was observed between α-galactosides, soyasapogenol B and lectin contents in beans.

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