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Effect of seed size and testa colour on saponin content of Spanish lentil seed

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Twenty cultivars of *Lens culinaris* Medik. were grown over 5 years in 18 provinces of Spain. Saponin content in the harvested seed ranged from 654 to 1269 mg kg^{-1}. Two saponins were detected and identified as soyasaponins I and VI. Changes observed in the saponin content were found to be dependent on both seed size and testa colour. Lentils of the subspecies *macrosperma* were found to have a significantly higher saponin content (1105 mg kg^{-1}) than seeds of the subspecies *microsperma* (929 mg kg^{-1}). The total saponin content of seeds with a brown testa was significant lower (800 mg kg^{-1}) than those with a beige or green testa (1106 and 1089 mg kg^{-1}, respectively). Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Lentils have made a significant contribution to the human diet since ancient times. There are two subspecies of lentils, *Lens culinaris* ssp. *macrosperma*, which is characterized by flat pods and large seeds, and *Lens culinaris* ssp. *microsperma*, which has small seeds and convex pods. The seeds show considerable variation in both testa and cotyledon colour. The subspecies *macrosperma* includes the so-called Chilean or yellow cotyledon types of lentil, while the subspecies *microsperma* includes the Persian lentils or red cotyledon types. Lentils, as other crop legumes, are highly nutritious, containing about 25% protein, 56% carbohydrate and 1.0% fat. They are one of the best and cheapest sources of vegetable protein and provide a good source of minerals. Although lentils are considered to be one of the most nutritious pulses, they do contain several antinutritional factors which could limit their consumption (Adsule *et al.*, 1989). One class of these is the saponins which are composed of a steroidal or triterpene aglycone linked to one, two or three saccharide chains of variable size and complexity via ester and ether linkages. This class of bioactive compound is present in many human foods, including legumes and root crops, and is also found in many medicinal herbs (Price *et al.*, 1987). Saponins are attracting considerable interest as a result of their diverse properties, both deleterious and beneficial (Fenwick *et al.*, 1991). They are toxic to fish (Dorsaz *et al.*, 1988) and inhibit the growth and sporulation of a wide range of fungi (Gestetner *et al.*, 1971). Retardation of growth by dietary saponins has been observed in livestock and laboratory animals (Cheeke, 1980), probably due to their bitter and astringent sensory characteristics in processed grain legume products (Price *et al.*, 1985). Among the better-known biological effects of saponins are their capacity to cause lysis of erythrocytes (Khalil & El-Adawy, 1994) and to make the intestinal mucosa permeable (Johnson *et al.*, 1986). Conversely, a beneficial lowering of plasma cholesterol levels in humans has also been attributed to saponins (Sidhu & Oakenfull, 1986), whilst some have been reported to exhibit anticancer activity (Konoshima *et al.*, 1992) and an inhibitory effect on the infectivity of human immunodeficiency virus *in vitro* (Nakashima *et al.*, 1989). However, each of these properties is related to specific saponin structures rather than to the class as a whole.

The possibility of producing lentil varieties, optimised for their end use and meeting nutritional requirements, whilst reducing undesirable components (e.g. the raffinose family of α-galactosides producing flatulence; Frias *et al.*, 1994), has become of increasing importance for the modern breeding programmes of this type of legume. However, very little is known about the level of saponins in lentils and the relationship between these bioactive compounds and morphological seed characteristics. Therefore, there is clearly a need to provide

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relevant information in order to allow selections and crosses to be made with the aim of improving the compositional quality of lentils.

The objective of this work was to examine differences in saponin content of 20 cultivars of lentil seed grown in Spain in relation to seed size and testa colour.

MATERIAL AND METHODS

Material

Seed from 20 cultivars of *Lens culinaris* Medik. were obtained from one crop of each cultivar grown between 1987 and 1992 in areas of 18 provinces of Spain.

All solvents used during the extraction process were of analytical reagent grade and redistilled before use.

Extraction of saponins

The ground sample (5 g) was mixed with sand (10 g) and extracted in a Soxhlet apparatus sequentially with chlorofom (200 ml, 16 h) and methanol (200 ml, 30 h). The methanol extract was evaporated to dryness *in vacuo* and redissolved in water (5 ml). The aqueous solution was eluted through a glass column dry-packed with SiO_2–C_{18} (20 g; JT Baker UK, Hayes, Middlesex, UK) which had previously been conditioned first with methanol (100 ml) and then with water (150 ml). The elution was carried out in two stages, first with water (150 ml) and then with methanol (150 ml) under a pressure of 10 psi (1 psi $\approx$ 6.9 kPa). The methanol fraction was evaporated to dryness under reduced pressure and the residue made up to 5 ml in methanol, corresponding to 1 g ml^{-1} starting material, ready for acid hydrolysis.

Fast atom bombardment-mass spectrometry (FAB-MS)

Aliquots of 5 μl of each methanol solution were added to a drop of glycerol on the FAB copper probe tip. Mass spectra were obtained using a Kratos MS 9/50TC mass spectrometer by bombardment of the sample with an ion beam of xenon produced by an Ion-Tech 11 NF atom gun operating at 9 kV (nominal). Positive and negative ion spectra were recorded using a UV galvanometer recorder.

Acid hydrolysis of saponin fractions

Two aliquots (1 ml) of each of the methanol solutions were evaporated to dryness and dried over phosphorus pentoxide in a vacuum desiccator overnight. The residues were refluxed for 3 h with acetyl chloride in methanol (5% v/v, 5 ml, a source of dry HCl). After cooling, the hydrolysates were neutralised with aqueous ammonium hydroxide (2 M) and evaporated to dryness. After redissolving in water (5 ml), the sapogenols were extracted with ethyl acetate (3×5 ml). The first 5 ml of ethyl acetate contained a standard of cholesteryl-n-decanoate (0.2 mg ml^{-1}). The combined ethyl acetate extracts were dried over anhydrous sodium sulphate, filtered and evaporated to dryness.

Gas chromatography of sapogenols

The residues equivalent to 1 g of defatted flour were redissolved in ethyl acetate (1 ml) and evaporated to dryness in a vial by a stream of nitrogen. After vacuum drying over phosphorus pentoxide for 12 h, the hydrolysate was derivatised by heating with bis(trimethylsilyl)trifluoroacetamide (100 μl) and pyridine (100 μl) in sealed vials (50°C, 20 min). Five microlitres of each sample were injected onto a Hewlett Packard 5890 Series II gas chromatograph fitted with a glass column (30 m×0.32 m i.d.) coated with DB-1, 0.25 μm film (J and W Scientific, Serial No. 1129125) and flame ionisation detector. The maximum oven temperature was 340°C, detector 300°C and injector 290°C, with helium as the carrier gas.

Gas chromatography-mass spectrometry (GC-MS) of sapogenols

Peak identity of the derivatised sapogenols was determined by co-elution with standards as well as by GC-MS. The column was the same as used for GC estimation of sapogenols above, and was coupled to a Kratos MS 30 mass spectrometer, operating at a source temperature of 250°C and a nominal ionising voltage of 70 eV.

Quantitative determination of saponins and sapogenols

Quantification was achieved using soyasaponin I isolated from pea seed (Price & Fenwick, 1984), against an internal standard of cholesteryl-n-decanoate. The relationship between the weight of soyasaponin I and the peak area ratio of soyasapogenol B with internal standard was a straight line with a correlation coefficient of 0.995.

Statistical methods

The data were subjected to standard one-way analyses of variance (unequal groups), using Minitab 8.21 Software (Macintosh version) to determine the statistical significance of differences between the sources of variation being examined.

RESULTS AND DISCUSSION

Saponins were detected in all the lentil seeds. Seed characteristics and total saponin content of the 20 cultivars of *Lens culinaris* Medik. grown in 18 different provinces of Spain are shown in Table 1.

Table 1. Cultivar, growing site, 100 seed weight, seed cotyledon and testa colour, and total saponin content of Spanish *Lens culinaris* Medik

Cultivar	Site weight	100 seed weight (g)	Cotyledon colour	Testa colour	Total saponin content (mg kg^{-1})
BG-1017	Granada	5.08	Yellow	Green	1245
BG-1023	Córdoba	6.16	Yellow	Beige	1269
BG-1048	Tenerife	2.40	Yellow	Beige	1045
BG-1055	Jaen	6.16	Yellow	Beige	1044
BG-1078	Palencia	5.48	Yellow	Beige	1080
BG-1119	Guadalajara	6.64	Yellow	Beige	1100
BG-1150	Zamora	6.76	Yellow	Green	1049
BG-1409	Albacete	6.92	Yellow	Beige	1269
BG-1415	Baleares	4.72	Yellow	Beige	1175
BG-1431	Valladolid	2.28	Yellow	Brown	654
BG-1482	Cuenca	6.52	Yellow	Green	910
BG-1805	Madrid	5.08	Yellow	Beige	1163
BG-4245	León	3.20	Yellow	Brown	771
BG-4249	Soria	5.60	Yellow	Beige	854
BG-4250	Burgos	2.36	Yellow	Green	1221
BG-8982	Salamanca	5.72	Yellow	Beige	1102
BG-11086	Guadalajara	3.56	Yellow	Beige	1028
BG-11095	Ciudad Real	4.45	Yellow	Beige	937
BG-16363	Las Palmas	1.28	Red	Brown	750
BG-16366	Las Palmas	2.64	Red	Brown	1023

The FAB-MS showed the presence of two saponins in the mixture with relative molecular masses of 942 and 1068. The common aglycone of these was confirmed to be soyasapogenol B by GC-MS. From its chromatographic behaviour, the former saponin was identified as soyasaponin I and the latter as soyasaponin VI (Fig. 1). Soyasaponin VI (Massiot *et al.*, 1992), also known as soyasaponin βg (Kudou *et al.*, 1993), contains a 2,3-dihydro-2,5-dihydroxy-6-methyl-4*H*-pyran-4-one at the C-22 position of soyasaponin I, and has been postulated to be the natural precursor of soyasaponin I (Massiot *et al.*, 1992).

It has been shown that lentil is a legume with a relatively low saponin content when compared to soybean, haricot or kidney bean, which have saponin levels of 6500, 4100 and 3500 mg kg^{-1}, respectively. Lentil has a level comparable to that of pea (1100 mg kg^{-1}; Price *et al.*, 1986), but a high level when compared to lupin, whose saponin content ranged from 379 to 740 mg kg^{-1} (Ruiz *et al.*, 1995).

Table 2 shows the relationship between lentil seed size and total saponin content. *Macrosperma* seeds (100 seed weight > 4.5 g) were found to have a significantly larger total saponin content (1105 mg kg^{-1}) than *microsperma* seeds (100 seed weight < 4.5 g), which showed a saponin level of 929 mg kg^{-1}.

Table 3 shows the effect of seed testa colour on the total saponin content of lentils. Although no significant differences were found between seeds with beige or green testas, whose saponin levels were 1106 and 1089 mg kg^{-1}, respectively, brown-coated lentils showed a highly significant lower saponin content (800 mg kg^{-1}).

The results reported here are in good agreement with those reported by Kim & Okubo (1993), who observed that the content of group B saponins, which have

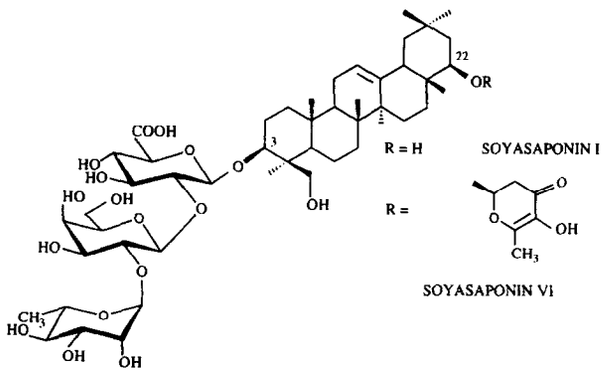

Fig. 1. Chemical structures of soyasaponin I and soyasaponin VI.

Table 2. Effect of seed size on the total saponin content of *Lens culinaris* Medik

Seed size	Number of cultivars	Total saponin content (mg kg^{-1})	
		Mean	Range
Subspecies *macrosperma*	12	1105[a]	854–1269
Subspecies *microsperma*	8	929[b]	654–1221

Macrosperma, 100 g seed > 4.5 g. *Microsperma*, 100 g seed < 4.5 g.
Different superscripts in a column indicate a significant difference ($P < 0.05$).

Table 3. Effect of seed testa colour on the total saponin content of *Lens culinaris* Medik

Seed testa colour	Number of cultivars	Total saponin content (mg kg^{-1})	
		Mean	Range
Beige	12	1106[a]	854–1269
Green	4	1089[a]	910–1245
Brown	4	800[b]	654–1023

Different superscripts in a column indicate a significant difference ($P < 0.05$).

soyasapogenol B as aglycone (Shiraiwa *et al.*, 1991), in soybeans showed significant differences depending on seed size. Also according to these authors, soybeans with yellow coats showed a higher saponin content than mixed and brown-coated seeds.

The work described here provides for the first time quantitative results on the effect of seed size and testa colour on saponin content of *Lens culinaris* Medik. This type of information should be useful in the screening and selection of low or high saponin-containing varieties of lentil as part of any future breeding programme for the manipulation of levels of these biologically active compounds.

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