Improvements in the High-Pressure Liquid Chromatographic Determination of Amino Sugars and α-Galactosides in Faba Bean, Lupine, and Pea

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Two HPLC systems used for the quantification of glucosides—vicine [2,6-diamino-5-(β-D-glucopyranosyloxy)-4(1*H*)-pyrimidinone] and convicine [6-amino-5-(β-D-glucopyranosyloxy)-2,4(1*H*,3*H*)-pyrimidinedione]—and α-D-galactosides (raffinose, stachyose, verbascose) present in faba beans are described. The silica-based HPLC supports used, a reversed C18-bonded phase and an amino-modified silica phase for glucoside and α-galactoside analyses, respectively, demonstrated good stability. The relative UV (λ 273 nm) response factors of glucosides to uridine (used as internal standard) have been determined (0.8 and 0.6 for vicine and convicine, respectively). Different pea and lupine cultivars have been screened for their α-galactoside contents too. The proposed method, simple, reliable and rapid (based on a unique extraction and purification procedure for both components), may offer a convenient alternative for routine analysis.

Faba bean (*Vicia faba* L.) has been reported as a valuable potential source of food quality protein (Davidson, 1973). The bean contains 28–30% high-lysine protein (Aykroyd and Doughty, 1964) and is considered as an alternative to high-cost soya bean meal for livestock and poultry feed. However, faba bean contains antinutritive factors like hemagglutinins (Allen et al., 1976), antitrypsic factors (Belitz et al., 1968), α-galacotosides of the raffinose family (Lineback and Ke, 1975; Cerning-Beroard and Filiatre, 1976; Vose et al., 1976), which are known to be indigestible and hence flatulent (Calloway and Murphy, 1968; Cristofaro et al., 1974; Fleming, 1981), and glucosides (vicine and convicine), which are believed to be responsible for an acute hemolytic disease known as favism (Lin and Ling, 1962; Mager et al., 1965).

The removal of these antinutritive factors by suitable processing techniques or the selection of cultivars with low levels would solve these problems. In the last years, some HPLC methods for quantitative analysis of α-galactosides (Macrae and Zand-Moghaddam, 1978; Quemener and Mercier, 1980; Wight and Datel, 1986) and glucosides (Marquardt and Fröhlich, 1981; Lattanzio et al., 1982; Quemener et al., 1982; Bjerg et al., 1985) have been described. As standards of vicine, convicine, and verbascose are not available, the accurate and routine determination of these compounds is questionable. The aim of the present work was to propose a simple, fast, and sensitive HPLC method routinely usable to estimate glucosides and α-galactosides. For this purpose, relative UV response factors of vicine and convicine to uridine (commercially available) have been determined, and different faba bean, pea, and lupine cultivars were screened for their glucoside and α-galactoside contents.

EXPERIMENTAL SECTION

Plant Materials. Inbred-line winter faba bean (*V. faba* L. var. minor) was obtained from INRA (Institut National de la Recherche Agronomique), Rennes, France. Other faba bean seeds were from UNIP (Union Nationale Inter Protéagineux)–ITCF (Institut Technique des Céréales et des Fourrages). Smooth pea (*Pisum sativum* L., var Koral and Karat from Sobotka, Poland, 1986, and var. Opal from Szelekewo, Poland, 1986) and lupine seeds (*Lupinus albus* L. from Lusignan, France, and *Lupinus angustifolius* L. and Lupin Kiev mutant from West Australia) were included in the study for comparative purposes.

Chemicals and Enzymes. HPLC-grade S acetonitrile was from Rathburn. Poly(ethylene glycol) 35 000 and 1,4-diaminobutane were from Merck. Sucrose and raffinose were obtained from Fluka. Stachyose was from Koch-Light. Uridine, β-glucosidase from sweet almonds (20 U/mg), and α-galactosidase from green coffee beans (10 U/mg) were from Boehringer Mannheim.

General Methods. Moisture contents were determined by drying at 130 °C for 2 h. Compositions were calculated on a moisture-free basis.

Extraction, Purification, and Quantification of Verbascose and Glucoside Standards. Verbascose was isolated by preparative HPLC of an 80% enthanol extract of smooth pea seed. The extract was purified by Carrez salts (Cerning-Beroard, 1975), treated with activated charcoal, and concentrated under vacuum at 40 °C. After filtration on a 3-μm Millipore filter, the sample was applied to a (4 × 50 cm) preparative HPLC column (Jobin et Yvon, France) packed with 200 g of Lichroprep NH_2 (25–40 μm, Merck) and eluted with 70–30 acetonitrile/water (flow rate about 50 mL/min). Detection was ensured by a R401 refractive index detector (Waters Associates). The fraction corresponding to the large peak eluted just after stachyose was collected, concentrated, and rechromatographed. The purity of isolated verbascose was determined by analytical chromatography under the same conditions as for α-galactoside quantitative analysis. Verbascose content was determined (i) by an enzymatic procedure according to the standard Boehringer method described for raffinose determination (Boehringer, 1980) and (ii) by an orcinol–sulfuric acid colorimetric procedure (Kesler, 1967; Tollier and Robin,1979) using a mixture of sucrose and galactose in a molar ratio 1/3 as standard.

Vicine and convicine were isolated by chromatography on preparative HPLC column (200 g of RSil C18 HL, 12–40 μm; Alltech Associates), from a concentrated faba bean 80% ethanol extract. After deproteinization, and concentration, the sample was injected on the column and eluted with ultrapure water (Millipore). Flux was monitored with both a differential refractometer and a UV III detector at 280 nm (Laboratory Data Control). Five successive runs were required for a complete purification

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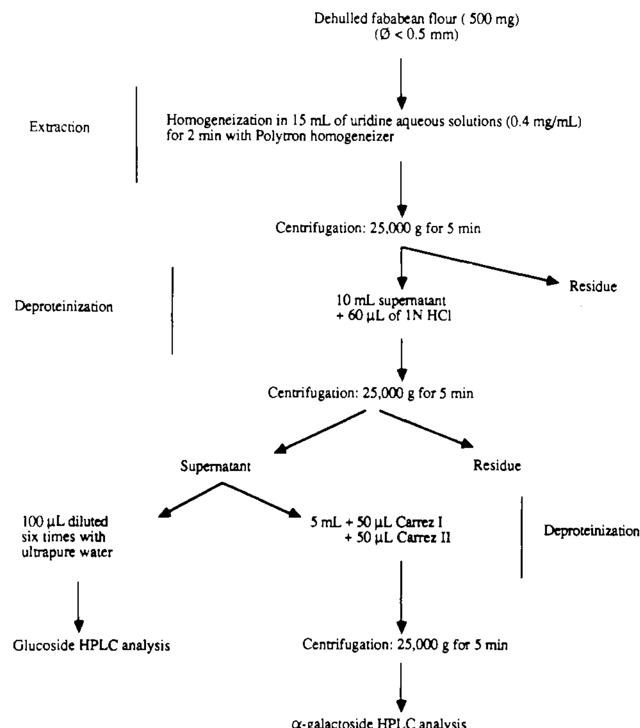

Figure 1. Scheme of the extraction and purification procedure.

of vicine and convicine. Purity was controlled by analytical chromatography. Vicine and convicine contents were determined (i) after β-glucosidase treatment (1 mg/2 mol of glucoside at 37 °C for 15 h), by standard Boehringer UV method for glucose determination (Boehringer, 1980), and (ii) by spectrophotometry of solutions in 0.1 N NaOH at 269 nm for vicine (Bendich and Clements, 1953) and at 273 nm for convicine (Bien et al., 1968).

Determination of Relative UV Response Factors. Relative UV response factor values of vicine and convicine were the means of 10 values obtained via the HPLC analysis of three independent reference aqueous mixtures (A–C). These sample mixtures, containing glucosides and uridine (internal standard) in respective linear concentration ranges, were freshly prepared and quantified separately. A wavelength of 273 nm was selected for HPLC detection. The accuracy of UV wavelength calibration of the UV detector was checked by comparison of the ratio value of uridine responses at two wavelengths (261/273 nm) obtained by HPLC Spectromonitor III (LDC) after a chromatographic run to the same ratio value determined with a Beckman DU-40 spectrophotometer. The relative response factors (RF) were calculated on a Shimadzu CR3A integrator.

Proposed Method. The proposed procedure is shown in Figure 1.

Extraction and Purification Procedure. Dehulled faba bean seeds were ground in an IkA-Universal M20 mill for 3 min to a particle size of less than 0.5 mm. A 500-mg quantity of sample was weighed in a 50-mL centrifuge tube, and 15 mL of aqueous uridine solution (0.4 mg/mL) was added. The tube contents were homogeneized for 2 min with a Polytron homogeneizer equipped with 10S Model axis and centrifuged at 25000*g* for 5 min. Portions of 10 mL of supernatant were transferred to another centrifuge tube, and 60 μL of 1 N HCl was added in order to precipitate most of the proteins at their isoelectric pH (pH_i ~4.2). The mixture was shaken and centrifuged at 25000*g* for 5 min. An aliquot of supernatant (100 μL) was diluted six times with ultrapure water and maintained at 4 °C until glucoside HPLC analysis. An other aliquot of supernatant (5 mL) was transferred in a centrifuge tube, and 50 μL of Carrez I (1%, v/v) and 50 μL of Carrez II (1%, v/v) were added. After centrifugation (25000*g*, 5 min) the supernatant was directly analyzed for α-galactoside content.

HPLC Analysis. The HPLC system consisted of a Waters Associates 590 programmable pump, an Erma ERC-3310 degasser, a Touzart/Matignon pulsation damper, and a Valco C6w injection valve equipped with 10- and 25-μL loops for glucoside and α-galactoside analyses, respectively.

Glucosides were analyzed on a Rosil C18 (3 μm, 15 cm × 0.46 cm; Alltech Associates) column packed in our laboratory by the slurry technique (Coq et al., 1975). This column was equipped with a 5 cm × 0.46 cm precolumn packed with RSil C18 HL (5 μm; Alltech Associates). Ultrapure water was eluent at a flow rate of 1 mL/min. The wavelength of the UV detector was adjusted to 273 nm (0.05 AUFS).

Oligosaccharides were analyzed on a Lichrospher Si60 4 μm, 25 cm × 0.46 cm) cartridge (Merck). Solvent was 53/47 acetonitrile/water containing 1,4-diaminobutane (0.02%) and poly(ethylene glycol) 35 000 (0.2%) (Praznik and Beck, 1984). The solvent mixture was degassed under vacuum for 5 min. Flow rate was 0.7 mL/min and the pressure about 2700 psi. The sensitivity of the RI detector (Erma ERC-7510) was set to 1 or $^1/_2 \times 10^{-5}$ RIU/FS, and RI temperature was maintained to 40 °C (column was just isolated from room-temperature fluctuations).

RESULTS AND DISCUSSION

Purity and Quantification of Standards. The chromatography of purified verbascose on an HPLC analytical column showed that this standard was only slightly contaminated by stachyose (<1.8%). Determinations of the amount verbascose in aqueous solution, performed on duplicates by enzymatic and colorimetric methods, were very similar (8.54 and 8.64 mg/mL, respectively). The purities of vicine and of convicine, estimated by analytical HPLC, were higher than 99.7 and 99.6%, respectively. The λ_{max} values of the purified vicine and convicine in 0.1 N NaOH were 267 and 273 nm, respectively, values very similar to those previously reported (Bendich and Clements, 1953; Bien et al., 1968). The quantitative determinations of these glucosides, performed in duplicate by both analysis methods, were in good agreement (170.9 and 175.6 μg/mL for vicine and 50.6 and 52.7 μg/mL for convicine by enzymatic and spectrophotometric procedures, respectively).

Extraction Procedure. α-Galactosides or glucosides are usually extracted by mixtures of alcohol and water (Cerning, 1970; Lineback and Ke, 1975; Vose et al., 1976; Macrae and Zand-Moghaddam, 1978; Fleming, 1981; Bien et al., 1973; Lattanzio et al., 1982; Bjerg et al., 1985).

These extraction procedures are time-consuming and tedious, and the extraction of verbascose is not always complete (Quemener et al., 1982). Glucoside extraction with trichloroacetic acid produces low yields (~0.30%) (Higazi and Reed, 1974). The use of a 25% (v/v) solution of acetic acid was successfully tried (Chevion and Navok, 1983): As the bonded stationnary phases used for glucoside and α-galactoside determination (C18, NH_2) are sensitive to acid pH, this extraction solvent could not be used without neutralization and desalting. Marquardt and Fröhlich (1981) claimed that vicine and convicine are less stable in acidic medium (5% perchloric acid) than in aqueous solution. NaOH solution (0.1 N) was used for glucoside extraction (Pitz and Sosulski, 1979; Quemener et al., 1982), but this extraction introduces sodium chloride

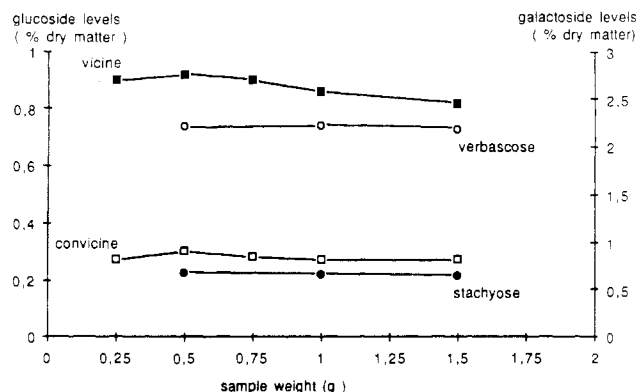

Figure 2. Extraction of glucosides and main α-galactosides of faba bean cotyledons as a function of sample weight.

by using alkali and acid steps to precipitate proteins to their isoelectric pH. Although sodium chloride does not absorb in the UV, it interferes with α-galactoside detection on the RI chromatogram (Quemener et al., 1982). In order to develop an efficient, simple, and rapid extraction, we tested water. For this purpose, glucosides and α-galactosides of various sample amounts (250 mg, 500 mg, 750 mg, 1 g, 1.5 g) were extracted with the same volume of water (15 mL). results (Figure 2) showed that extraction procedure was efficient up to 750 mg for vicine and 1.5 g for convicine, respectively. Concerning verbascose, which is the main α-galactoside in faba bean and the less soluble component, extraction was efficient over the range tested. In order to propose an unique procedure suitable for both glucoside and α-galactoside detemination, sample weight was fixed at 500 mg (wet basis). The precipitation of proteins at their isoelectric pH was sufficient to allow subsequent analysis of glucosides on C18 phase, but for α-galactoside determination, an additional deproteinization step by Carrez salts was necessary to avoid protein precipitation on the analytical column. With this aim, the minimum amount of Carrez salts was selected (1%, v/v). A mixture of 50/50 ethanol/water (after concentration to dryness under vacuum (40 °C) of an aliquot of 1 mL of extract) may also be used. Carrez salts were chosen for the rapidity of procedure.

Justification of HPLC Methods Used. *Glucoside Analysis.* Pure water as eluent and an efficent stationary phase have been used in order to have a stable and simple chromatographic system. under these conditions, elution times were higher than those of Marquardt and Fröhlich but our system dos not need column washing and column equilibration. Moreover, our eluting time may be reduced by increasing the flow rate. In order to determine relative UV factors of vicine and convicine, cytosine, cytidine, adenosine, 2-desoxythymidine, uridine monophosphate, and uridine were tested as internal standards. Uridine was found suitable. This component is not present in faba bean extract; it is commercially available, is freely water-soluble, absorbs in the UV (λ_{max} 261 nm), and is well separated from vicine and convicine by our HPLC conditions (Figure 3). The relative UV response factors of vicine and convicine to uridine, measured at λ 273 nm on three reference mixtures freshly quantified, are given in Table I. RF values were 0.803 and 0.601 for vicine and convicine (means of 10 analyses) with reproducibilities of 1.1% and 1.5%, respectively. The same determination, performed on solution B, using the Spectromonitor 3000 (LDC) and another column was in very good agreement (0.813 and 0.591, respectively). The ratio values of uridine responses at two wavelengths (261/273 nm) were 1.43 and 1.40 with the HPLC detector and the Beckman DU-40

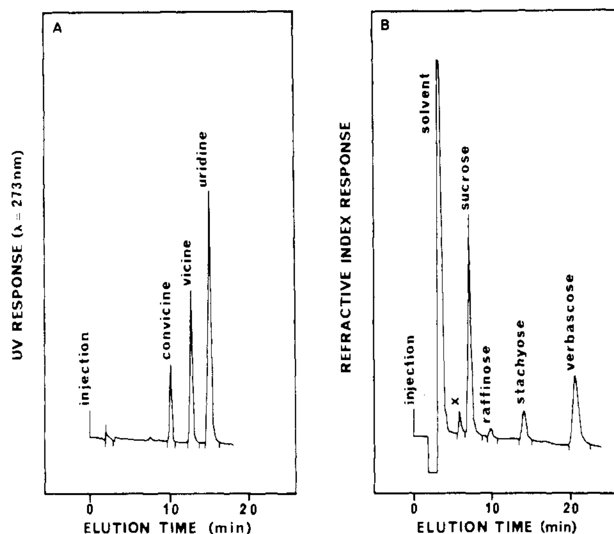

Figure 3. High-pressure liquid chromatograms of glucosides (A) and α-galactosides (B) from an aqueous extract of an inbred line winter faba bean: (A) glucosides analyzed on a Rosil C18 3-μm (15 cm × 0.46 cm) column, water as solvent (flow rate 1 mL/min); (B) α-galactosides chromatographed on a Lichrospher Si60 4-μm (25 cm × 0.46 cm) cartridge, 53/47 acetonitrile/water containing 1,4-diaminobutane (0.02%) and poly(ethylene glycol) 35000 (0.2%) as solvent (flow rate 0.7 mL/min).

Table I. Relative UV Response Factors of Vicine and Convicine to Uridine, Measured at 273 nm, via the HPLC Analysis of Three Independent Aqueous Mixtures (A–C) (See Figure 3A for Chromatographic Conditions)

	mixture	vicine	convicine
rel UV response factors	A	0.82	0.62
		0.79	0.60
		0.78	0.60
		0.81	0.61
		0.80	0.60
		0.81	0.59
	B	0.82	0.60
		0.80	0.59
		0.80	0.58
	C	0.80	0.62
mean, m		0.803	0.601
std dev, s		0.0125	0.0129
95% confidence int for μ based on t distribn, $ts/n^{1/2}$		0.009	0.009
reproducibility, $ts/n^{1/2} \times 100/m$		1.1%	1.5%

spectrophotometer, respectively, indicating accurate wavelength calibration of the UV detector used. The other main advantage of this HPLC system was the great efficiency and the good stability of the stationary phase. Theoretical plate number (N), measured on the uridine peak, was about 3500 with a new column. N decreased to 2916 after 50 analyses of faba bean samples with very good values of resolution (in the same time, the convicine and vicine peaks resolution decreased from 4.5 to 2.7). Finally, the chromatogram of a faba bean extract treated with β-glucosidase showed complete disappearance of the two main peaks corresponding to vicine and convicine. This result confirmed the identity of these components and indicated the absence of any coeluting substances.

α-Galactoside Analysis. HPLC α-galactoside determination is usually performed on 5-μm amino-bonded stationary phases with 70/30 acetonitrile/water as eluent (Macrae and Zand-Moghaddam, 1978; Quemener and Mercier, 1980). The main inconveniences of this chromatographic system are the high cost and the short life of amino-bond phases and the great consumption of organic solvent. Wight and Datel (1986) have proposed a

Table II. Reproducibility and Repeatability of HPLC Assay for the Determination of Glucosides and Oligosides in Inbred-Line Winter Faba Bean (See Figure 3 for Chromatographic Conditions)

	vicine	convicine	sucrose	raffinose	stachyose	verbascose
mean, m (10 extrns of same sample)	0.92	0.30	2.96	0.08	0.68	2.21
std dev, s	0.021	0.009	0.186	0.008	0.051	0.140
95% confidence int, $ts/10^{1/2}$	0.015	0.006	0.133	0.006	0.036	0.100
reproducibility, $ts/10^{1/2} \times 100/m$	1.6%	2.1%	4.5%	6.9%	5.4%	4.5%
mean, m (10 injectns of same extr)	0.92	0.30	3.17	0.08	0.69	2.31
std dev, s	0.012	0.006	0.050	0.009	0.044	0.080
95% confidence int, $ts/10^{1/2}$	0.009	0.004	0.036	0.007	0.031	0.060
repeatability, $ts/10^{1/2} \times 100/m$	1.0%	1.5%	1.1%	7.1%	4.5%	2.5%

chromatographic system with an RP-18 column using distilled water as the mobile phase, but verbacose was coeluted with raffinose. Verbascose was completely separated when 1.5 M ammonium sulfate was used as the eluent. However the use of high salt concentrations was not convenient for routine analysis and shortened column life. A valuable alternative chromatographic system may be provided by an amine-impregnated silica column (Aizetmüller, 1978; Praznik and Beck, 1984) using an acetonitrile/water amine-modified mixture as mobile phase and 3–4-μm particle size. For this purpose we have tested three stationary phases bonded or nonbonded with amino groups: 3-μm NH_2 Spherisorb (Harwell AEC), 3-μm NH_2 Rosil (Alltech Associates), and 4-μm Si 60 Lichrospher (Merck). In order to decrease the proportion of acetonitrile in the mobile phase, we tested 25 cm × 0.46 cm columns. The better efficiency was obtained with the 4-μm Lichrospher cartridge using 53/47 acetonitrile/water amine modifier mixture (0.02% of amine in solvent) as mobile phase. With a flow rate of 0.7 mL/min, elution times were approximately the same as with the 5-μm phase (flow rate 2 mL/min). Moreover, the efficiency loss was only about 11% after 50 chromatographic runs in the described system (about 25% with amino-bonded phases in the same time) and the resolution values were always very good (4.7 and 4.3, respectively, in the same time for the stachyose and verbascose peaks resolutions). Finally, under present conditions, glucosides were eluted in the void volume and did not interfere with oligosaccharide peaks as they did with 70/30 acetonitrile/water on 5-μm amino-bonded phase (Quemener et al., 1982).

Characteristics of HPLC Methods. *Linearity.* The ratios of glucoside areas to internal standards areas obtained on different standard solutions were linear for both vicine and convicine over the range tested (0–200 and 0–120 μg/mL, respectively) (Figure 4) with correlation coefficients of 0.99. RI response of purified verbascose, based on area measurements, was the same as the RI response of standard stachyose. So, this available standard may be used for verbascose quantitative analysis.

Reproducibility and Repeatability. The reproducibility of the method (extraction, purification, HPLC analysis) was calculated from analyses of 10 extracts of the same faba bean sample (Table II). The reproducibility was better for glucosides (internal standard method) than for oligosaccharides (external standard method). After various attempts (maltotriose, isomaltotriose, lactose, trehalose, cellobiose), isomaltotriose was the better internal standard. However, for routine use, in view of its high price, it seemed unsuitable. Moreover, differences of reproducibility values observed resulted from the best stability of the chromatographic system used for glucoside analysis. As a matter of fact, the repeatability (calculated from 10 analyses of the same extract; Table II) was good for glucosides (1.0 and 1.5% for vicine and convicine, respectively) and acceptable for α-galactosides. In all cases, except for raffinose, which was present at low levels (about 0.08%),

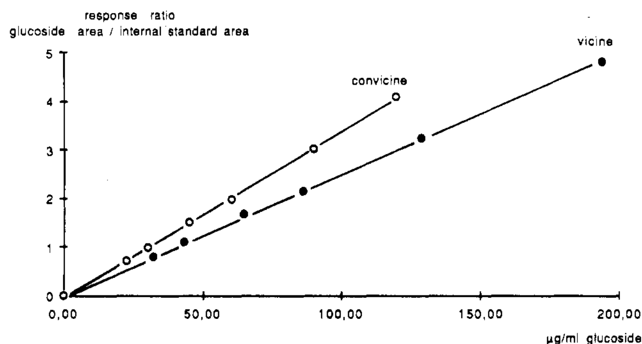

Figure 4. Calibration curves of an HPLC assay for vicine and convicine. Response ratios are plotted against glucoside concentration in the standard mixture. Chromatographic conditions are the same as in Figure 3A.

repeatability was lower than reproducibility. Glucoside repeatability were similar to those obtained by Marquardt and Fröhlich (1.2–2.1% and 1.5–2.5% for vicine and convicine, respectively) and better than values resulting from a previous study performed by the external standard method (2.5 and 3.3%, respectively; Quemener et al., 1982) and better than those of Pitz and Sosulski (1979) (2.6 and 2.9%, respectively).

Sensitivity. In order to test the sensitivity of the glucoside method, a protein isolate from Ascott cultivar faba bean flour (Gueguen et al., 1980) and the related spun proteins (Culioli and Sale, 1981) were examined for their glucoside contents. Vicine and convicine were detected at 2×10^{-2}% and 8×10^{-3}%, respectively, for the protein isolate and 2×10^{-5}% and 9×10^{-6}% for the spun proteins (glucoside levels were decreased by a factor of 1000 after the spinning process). Such very low levels in spun proteins corresponded to concentrations of 0.04 and 0.02 μg/mL for vicine and convicine, respectively, in the aqueous extract. The limit of detection for these glucosides was approximately 0.01 μg/mL (10^{-1} ng injected, 0.001 AUFS).

Application of the Method to Various Leguminous Seeds (Faba Beans, Peas, Lupines). Taking advantage of HPLC analysis of both glucoside and α-galactosides from the same extract, we have investigated different cultivars of faba beans. Results are shown in Table III. The relative distributions of both components were somewhat different from winter to spring varieties. Winter cultivars from the North of France (talo and alto) had the highest convicine levels (about 0.42 and 0.36%, respectively). The spring variety, ascott, had the lowest glucoside content (about 0.65%). A winter cultivar from the South of France, bourdon, had intermediate contents. For the same variety (talo), the extreme differences were about 5.7% for vicine and 17% for convicine. Our results were in good agreement with those of Pitz et al. (1981) (0.44–0.82% and 0.13–0.64% for vicine and convicine, respectively), of Marquardt and Fröhlich (1981) (0.53 and 0.28%), and of Bjerg et al (1985) (0.51–0.80% and

Table III. Glucoside and Sugar Distribution in Faba Bean Seed Cotyledons (Percent Dry Matter, Means of Duplicate Analyses) (Data, within Cultivars, Correspond to Different Seed Samples; See Figure 3 for Analysis Chromatographic Conditions)

cultivars	vicine	convicine	sucrose	raffinose	stachyose	verbascose
alfred	0.55	0.20	1.65	0.05	0.55	2.20
	0.66	0.23	1.85	0.05	0.55	1.90
	0.62	0.22	1.95	0.05	0.55	2.00
ascott	0.50	0.14	1.80	0.05	0.50	2.15
	0.50	0.16	2.00	0.05	0.45	2.10
bourdon	0.60	0.18	2.00	0.05	0.55	2.00
alto	0.52	0.36	2.20	0.10	0.65	2.20
	0.53	0.36	2.30	0.05	0.60	2.15
talo	0.51	0.43	2.45	0.10	0.60	2.15
	0.53	0.36	2.25	0.10	0.65	2.20
	0.51	0.42	2.25	0.05	0.60	2.15
	0.54	0.43	2.30	0.05	0.65	2.15

Table IV. α-Galactoside Composition of Pea Seed Cotyledons (Percent Dry Matter) (See Figure 3B for Analysis Chromatographic Conditions)

cultivars	extraction procedure[a]	raffinose	stachyose	verbascose
koral	a	0.40	1.70	1.90
	b	0.40	1.60	2.60
	c	0.45	1.70	2.60
opal	a	0.40	1.70	2.00
	b	0.45	1.80	2.60
	c	0.60	1.90	2.70
karat	a	0.35	1.80	2.10
	b	0.30	1.70	3.00
	c	0.45	1.85	3.20

[a] Key: a, 80% ethanol (Cerning, 1970); b, absolute ethanol + water (Tanaka et al., 1975; Kedzior, 1983); c, water (proposed method).

0.23–0.37%), but in all cases they were higher than the results of Chevion and Navok (1983) (total glucoside level of about 0.5%).

Concerning α-galactoside contents, similar differences were obtained between winter and spring cultivars. Winter cultivars had the highest levels. These results are similar to those obtained on five faba bean samples: sucrose, 1.5–2.3%, raffinose, 0.1–0.4%; stachyose, 0.5–0.8%; verbascose, 1.8–2.9% (Mercier, 1979).

The proposed method has also been applied to α-galactoside determinations in pea and lupine dehulled seeds. For comparative purposes, two other extraction procedures were performed on pea flours prior to HPLC analysis: extraction with 80% ethanol (Cerning, 1970) and extraction with absolute ethanol followed by water (Tanaka et al., 1975; Kedzior, 1983). Results are shown in Table IV. Raffinose and stachyose amounts determined by the different extraction methods were similar. On the other hand, verbascose amounts were in good agreement for the three varieties after both aqueous extractions when they were higher than amounts determined after 80% ethanol procedure (about 25% higher). These differences, observed in a previous study (Quemener et al., 1982), revealed that 80% ethanol does not completely extract verbascose. In Table V are summarized results of α-galactoside determination of lupine seeds. The oligosaccharide compositions are quite characteristic of the species studied. *L. angustifolius* seeds show the lowest amounts of stachyose (3.6–4.9%) and the highest verbascose levels (1.3–1.9%). These results are in good agreement with those of Macrae (3.5–3.8% and 1.2–1.9%, respectively). *L. albus* varieties have the highest amounts of stachyose (6.5–8.3%). These values are higher than those determined by Macrae on the same species (3.5–4.3%). This observation is true for sucrose, raffinose, and verbascose amounts too, so that total oligosaccharide amounts of french cultivars of *L. albus* are approximately double those of Australian varieties (10.5–14.4% and 5.4–6.7%, respectively). We have no explanation for this discrepancy. On the other hand, as α-galactosides of raffinose family have influence on flatus formation, this discrepancy might explain the differences of nutritional performances of french and australian pigs.

CONCLUSION

The proposed HPLC method determine glucosides and α-galactosides in faba beans from the same water extract. An extraction and purification procedure, rapid and efficient for these both components, was developed. We have determined the relative UV response factors of vicine and convicine to a convenient internal standard (uridine). The use of these factors (0.8 and 0.6 for vicine and convicine, respectively) allows, after identification, quantification of these glucosides by HPLC under the proposed conditions. The extraction and purification procedure is efficient for α-galactoside determination of pea and lupine seeds too. The proposed method, simple, reliable, and rapid may offer

Table V. Oligosaccharide Composition of Lupine Seed Cotyledons (Percent Dry Matter) (See Figure 3B for Analysis Chromatographic Conditions)

species	cultivar	sucrose	raffinose	stachyose	verbascose
L. angustifolius	Chittick	4.00	0.65	3.55	1.30
	Danja	3.50	0.55	4.40	1.50
	Marri	2.80	0.55	3.85	1.40
	Illyarie	3.90	0.80	4.85	1.90
	Wandoo	4.60	0.75	4.20	1.40
L. albus	Lutop 86	2.30	0.55	6.85	0.70
	LC 168 86	2.70	0.60	7.10	0.90
	LC 8 86	3.50	0.60	7.00	1.00
	Lucrop	3.80	0.50	7.85	1.30
	Lucky	5.80	0.65	6.55	0.60
	LC 32 86	3.50	0.50	7.55	1.00
	LC 164 86	4.50	0.70	8.30	0.90
	Lublanc 86	3.80	0.70	7.85	1.00
L. kiev mutant		2.80	0.60	6.40	0.50

a convenient alternative for routine analysis.

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Registry No. Vicine, 152-93-2; convicine, 19286-37-4; raffinose, 512-69-6; stachyose, 470-55-3; verbascose, 546-62-3; sucrose, 57-50-1.

LITERATURE CITED

Aizetmüller, K. *J. Chromatogr.* **1978**, *156*, 354.
Allen, A. K.; Desai, N. N.; Neuberger, A. *Biochem. J.* **1976**, *155*, 127.
Aykroyd, W. R.; Doughty, G. *Legumes in Human Nutrition*; FAO: Rome, 1964; FAO Nutritional Studies No. 19.
Belitz, H D.; Wassner, H. P.; Weder, J. *Z. Lebensm. Unters. Forsch.* **1968**, *137*, 211.
Bendich, A.; Clements, G. C. *Biochem. Acta* **1953**, *12*, 462.
Bien, S.; Salemnik, G.; Zamir, L.; Rosenblum, M. *J. Chem. Soc.* **1968**, 496.
Bien, S.; Amith, D. L. ; Ber, M. *J. Chem. Soc.* **1973**, 1089.
Bjerg, B.; Norgaard Knudsen, J. C.; Olsen, O.; Poulsen, M. H.; Sorensen, H. *Z. Pflanzenzuecht.* **1985**, *94*, 135.
Boehringer Mannheim GmbH *Methods of Enzymatic Food Analysis*; Boehringer Mannheim: 1980.
Calloway, D. M.; Murphy, E. L. *Ann. N.Y. Acad. Sci.* **1968**, *150*, 82.
Cerning, J. Ph.D. Dissertation, Unversity of Lille, Lille, France, 1970.
Cerning-Beroard, J. *Cereal Chem.* **1975**, *52*, 431.
Cerning-Beroard, J.;Filiatre, A. *Cereal Chem.* **1976**, *53*, 968.
Chevion, M.; Navok, T. A. *Biochemistry* **1983**, *128*, 152.
Coq, B.; Gonnet, C.; Rocca, J. L. *J. Chromatogr.* **1975**, *106*, 249.
Cristofaro, E.; Mottu, F.; Wuhrmann, J. J. *Sugars in Nutrition*; Sipple, H. L., McNutt, K. W., Eds.; Academic: New York, 1974; p 313.
Culioli, J.; Sale, P. *J. Texture Stud.* **1981**, *12*, 335.
Davidson, *J. Br. Poult. Sci.* **1973**, *14*, 557.
Fleming, S. E. *J. Food. Sci.* **1981**, *46*, 794.
Gueguen, J.; Quemener B.; Valdebouze, P. *Lebensm.-Wiss. Technol.* **1980**, *14*, 72.
Higazi, M. I.; Reed, W. W. C. *J. Agric. Food Chem.* **1974**, *22*, 570.
Kedzior, Z. M. Ph.D. Dissertation, University of Poznan, Poznan, Poland, 1983.
Kesler, R. B. *Anal. Chem.* **1967**, *39*, 1416.
Lattanzio, V.; Bianco, V. V.; La Fiandra, D. *Experientia* **1982**, *38*, 789.
Lin, J. Y.; Ling, K. H. *J. Formosan Med. Assoc.* **1962**, *61*, 490.
Lineback, D. R.; Ke, C H. *Cereal Chem.* **1975**, *52*, 334.
Macrae, R.; Zand-Moghaddam, A. *J. Sci. Food Agric.* **1978**, *29*, 1083.
Mager, J.; Glaser, G.; Razin, A. *Biochem. Biophys. Res. Commun.* **1965**, *29*, 235.
Marquardt, R. R.; Fröhlich, A. A. *J. Chromatogr.* **1981**, *208*, 373.
Mercier, C. *Les Matières Premières et Alimentaires des Volailles*; INRA, Station de Recherches Avicoles: Paris, 1979; pp 79–90.
Pitz, W. J.; Sosulski, F. W. *Can. Inst. Food Sci. Technol. J.* **1979**, *12*, 93.
Pitz, W. J.; Sosulski, F. W.; Rowland, G. G. *J. Sci. Food Agric.* **1981**, *32*, 1.
Praznik, W.; Beck, R. H. F. *J. Chromatogr.* **1984**, *303*, 417.
Quemener, B.; Mercier, C. *Lebensm.*-Wiss. *Technol.* **1980**, *13*, 7.
Quemener, B.; Gueguen, J.;; Mercier, C. *Can. Inst. Food Sci. Technol. J.* **1982**, *15*, 109.
Tanaka, M.; Thananunkul, D.; Lee, T. C.; Chichester, C. O. *J. Food Sci.* **1975**, *40*, 1087.
Tollier, M. T.; Robin, J. P. *Ann. Technol. Agric.* **1979**, *28*, 1.
Vose, J. R.; Basterrechea, M. J.; Gorin, P. A. J.; Finlayson, A. J.; Youngs, C. G. *Cereal Chem.* **1976**, *53*, 928.
Wight, A. W.; Datel, J. M. *Food Chem.* **1986**, *21*, 1.

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Rapid Extraction and Gas–Liquid Chromatographic Determination of *d*-Phenothrin in Aqueous Formulations

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A rapid method for extraction and quantitative determination of *d*-phenothrin in aqueous formulations is described. Aliquots of the formulations are mixed with aqueous sodium chloride and acetonitrile and then partitioned with toluene. Interferences are removed by adsorption chromatography on a silica gel column. The *d*-phenothrin is eluted from the column with a mixture of 3% ethyl acetate in pentane. The amount of *d*-phenothrin is determined by gas–liquid chromatography without the need to make sample dilutions by use of the linear flame ionization detector. The minimum detectability of this procedure was 0.10 ppm with a linearity of response for four decades.

Bry et al. (1980) reported on the effectiveness of pyrethroid combination sprays containing *d*-phenothrin [Sumithrin, (3-phenoxyphenyl)methyl *cis,trans*-(+)-2,2-dimethyl-3-(2-methyl-1-propenyl)cyclopropanecarboxylate] and tetramethrin [Neo-Pynamin, (1,3,4,5,6,7-hexahydro-1,3-dioxo-2*H*-isoindol-2-yl)methyl 2,2-dimethyl-3-(2-methylpropenyl)cyclopropanecarboxylate]. The pyrethroid combination in aqueous spray formulations effectively protected woolen cloth against damage by larvae of the black carpet bettle, *Attagenus unicolor* (Brahm), the furniture carpet beetle, *Anthrenus flavipes* (LeConte), and the webbing clothes moth, *Tineola bisselliella* (Hummel). These formulations were effective as direct-contact sprays against both larvae and adults. In addition, most of the adult carpet beetles or clothes moths that came in contact with the treated fabric were killed or knocked down. Subsequently, Bry et al. (1981, 1983) reported that pressurized solvent-based *d*-phenothrin formulations alone were effective in protecting woolen cloth against feeding damage by larvae of black and furniture carpet beetles and the webbing clothes moth.

Because *d*-phenothrin showed promise as a useful woolen cloth protectant upon application from aqueous

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