JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Compositional Variations for α-Galactosides in Different Species of Leguminosae, Brassicaceae, and Barley: A Chemotaxonomic Study Based on Chemometrics and High-Performance Capillary Electrophoresis

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The contents of raffinose family oligosaccharides (RFO) and sucrose in *Brassica*, *Lupinus*, *Pisum*, and *Hordeum* species were investigated by chemometric principal component analysis (PCA). *Hordeum* samples contained sucrose and raffinose, and *Brassica* samples all contained sucrose, raffinose, and stachyose. In addition to these, the *Pisum* samples contained verbascose and the *Lupinus* samples also contained ajugose. High stachyose and low ajugose contents were found in *Lupinus albus* in contrast to *Lupinus angustifolius*, having low stachyose and high ajugose contents. *Lupinus luteus* had average stachyose and ajugose contents, whereas large amounts of verbascose were accumulated in these seeds. *Lupinus mutabilis* had high stachyose and low ajugose contents, similar to the composition in *L. albus* but showing higher raffinose content. The *Brassica* samples also showed compositional RFO variations within the species, and subgroup formations were discovered within the investigated *Brassica napus* varieties. PCA results indicated compositional variations between the investigated genera and within the various species of value as chemotaxonomic defined parameters and as tools in evaluations of authenticity/falsifications when RFO-containing plants are used as, for example, feed and food additives.

KEYWORDS: Leguminosae; Brassicaceae; high-performance capillary electrophoresis; α-galactosides; raffinose family oligosaccharides; sucrose; raffinose; stachyose; verbascose; ajugose; chemometrics; principal component analysis

INTRODUCTION

Raffinose family oligosaccharides (RFO), also named α-galactosides, are nonreducing carbohydrates consisting of α-1−6-linked galactopyranosyl units linked to C-6 of the glucose moiety of sucrose. The RFOs are indigestible by alimentary enzymes of monogastric animals but pass into the lower gut, where they are fermented into short-chain fatty acids (SCFA). One of the effects of the fermentation is an increase of the biomass within the gut by stimulation of bacterial growth and thus contribution to a laxative effect by eventual excretion of the intestinal bacteria in feces (*1*). According to the newly published AACC dietary fiber definition (*2*), plant components with physiochemical properties such as the above-mentioned are include in the dietary fiber fraction.

The inclusion of high levels of protein-rich legume seeds in feeds for monogastric animals is hindered by the presence of antinutritional compounds such as protease inhibitors, lectins, phenolic compounds, phytates, and α-galactosides, of which some have been shown to reduce the availability of nutrients and cause growth inhibition (*3*). It is furthermore generally acknowledged that intake of a diet containing high amounts of legumes commonly results in intestinal discomfort and flatulence formation (*4*−*6*). This is at least partly due to the content of RFOs (*7*), and the flatulence is considered, by legume researchers, growers, processors, and consumers, the single most important factor that discourages people from eating more legume seeds, despite their being a good source of protein in the diet (*8*). Nevertheless, oligosaccharides are widely used as functional food ingredients in Japan and are added to such products as soft drinks, cookies, cereals, and candies (*9*); within the past decade there has been an increasing tendency to add indigestible oligosaccharides to foods and beverages, and the functional properties and assumed health benefits of RFOs have increased the interest for nutritional and functional utilization of these compounds in the novel food industries (*10*). New methods for the isolation of α-galactosides have recently been developed (*10*, *11*), and it is likely that the addition of α-galactosides to functional foods and drinks will increase

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10.1021/jf040471v CCC: $30.25
Published on Web 06/09/2005

within the next few years, not only in Japan but in the Western part of the world as well.

Chemotaxonomic determination of the origin of plant substances added to foodstuffs is for the various reasons mentioned above a desirable analytical tool. The level and composition of antinutritional compounds may vary for different legumes (*8*, *12*−*21*), and compositional variations of RFOs in different pea varieties have been demonstrated by Donangelo et al. (*22*). The purpose of this study is to identify patterns in the α-galactoside content of different *Lupinus* and *Brassica* species as a prestudy for the development of chemotaxonomic profiles for the identification of sources of low molecular weight (LMW) dietary fibers added to feeds, foods, and beverages.

MATERIALS AND METHODS

Plant Material. The examined lupine samples comprised four different *Lupinus* species: white lupine (*Lupinus albus* L.) from Frauenhofer Institute, Freising, Germany; blue or narrow-leafed lupine (*Lupinus angustifolius* L.) and pearl lupine (*Lupinus mutabilis*), kindly provided from the plant breeding group at RVAU (Højbakkegaard, Tåstrup, DK); and yellow lupine (*Lupinus luteus* L.) from Trifolium Silo A/S, Roskilde, Denmark. For all species except *L. mutabilis* L., several varieties were included in the study. The *L. albus* L. varieties were represented by the cultivars (cv.) Amiga, Feli, Nelly, Minori, CH-304, and Weibit, whereas the cv. 9909, E101, LAG-24, Sonet (W26), and Zubra represented the *L. angustifolius* L. varieties. The *L. luteus* L. varieties comprised Juno (Juno, Juno 96, and Juno 97: three different harvest years resulting in various degrees of ripeness due to differences in growth conditions), and Teo. *L. mutabilis* L. cv. 91 was the only variety of the pearl lupine. The Brassicaceae samples analyzed in this study comprised the three species *Brassica napus* L., *Brassica nigra* L., and *Brassica campestris* L. *B. campestris* L. and *B. nigra* L. were represented by only a single variety for each. The *B. napus* L. samples comprised 18 cultivars (Apex, Canary, Capitol, Contact, Escort, Express, Fortress, Gemini, Herald, Lipton, Lirajet, Madrigal, Mascot, HEAR, Pollen, Pronto, Synergy, and Tradition). All *Brassica* samples were obtained from The Scottish Agricultural College, Aberdeen, Scotland. Besides the *Lupinus* and *Brassica* samples a single barley variety, *Hordeum vulgare* L. cv. Vega (Trifolium Silo A/S), and one field pea cultivar, *Pisum sativum* L. cv. Bodil (Dæhnfeldt, Odense, Denmark), were included in the study. The symbol "nn" (no name), when used in this paper, indicates that the name of the variety is unknown to the authors.

Chemicals and Reference Compounds. Pyridine-2,6-dicarboxylic acid (PDC, dipicolinic acid), maltitol, methyl α-D-glucopyranoside (MGP), sucrose, raffinose, stachyose, and column materials for ion-exchange chromatography (Sephadex CM-25 H^+, Dowex 50WX8 H^+ 200−400 mesh, and Dowex 1X8 acetate 200−400 mesh) were obtained from Sigma Chemical Co. (St. Louis, MO). Cetyltrimethylammonium bromide (CTAB) was obtained from Fluka (Buchs, Switzerland). Other chemicals were of analytical reagent grade and obtained from various manufacturers. Galactinol, isolated from sugar beet, was kindly donated from Maribo (Danisco) Seeds A/S, Holeby, Denmark, and verbascose was isolated from peas using paper chromatography and preparative HPLC. Water was purified in a Milli-Q system (Millipore, Bedford, MA).

Sample Preparation and HPCE Analysis. The samples were prepared and analyzed by HPCE with indirect UV detection following the procedure described by Andersen et al. (*23*). The reference sample of oligosaccharides included sucrose and three α-galactosides of the raffinose family (raffinose, stachyose, and verbascose) together with two internal standards: maltitol and MGP. Reference compounds were dissolved in separation buffer in concentrations between 2.7 and 3.2 mg/mL of each.

Crushed seed material (0.5 g) was added to 100 μL of internal standard mixture consisting of 125 mM maltitol and 125 mM MGP prior to oligosaccharides extraction according to a standard procedure using Ultra Turrax T 25 homogenization (Janke & Kunkel, Staufen, Germany) in 2 × 3 mL of boiling methanol/water (7:3) for 2 × 2 min (*24*). The supernatants obtained after intermediary centrifugation at 2000*g* for 2 min were pooled and evaporated to dryness and redissolved in 5 mL of water. Group separation by ion-exchange chromatography according to the principles described by Bjerg et al. (*25*) and Sørensen et al. (*24*) was performed on 3.0 mL of water-soluble extracts, using a three-column system based on (A) Sephadex CM-25 H^+, (B) Dowex 50WX8 H^+ 200−400 mesh, and (C) Dowex 1X8 acetate 200−400 mesh. After application of the sample (6 × 0.5 mL), the material was allowed to pass into the column material, and the columns were then washed with 2 × 5 mL of water. The aqueous effluent was evaporated to dryness and redissolved in 200 μL of water, and 100 μL of the solution was evaporated to dryness and redissolved in separation buffer before HPCE analysis.

HPCE Analysis. A Hewlett-Packard HP^{3D} CE capillary electrophoresis system (Hewlett-Packard, Waldbronn, Germany) equipped with diode array detector was used for this study. The data processing was carried out by use of a HP Vectra 5/100 MHz Pentium with HP Chemstation v. 6.01. The capillary used for analyses was an 800 mm × 0.05 mm i.d. fused silica capillary. UV detection at 350 nm with a reference at 275 nm (*26*) was performed on-column at a position 700 mm from the injection end. Samples were introduced from the cathodic end of the capillary by vacuum injection for 5 s at 5 kPa. The separation buffer contained 20 mM PDC as background electrolyte, 50 mM sodium borate decahydrate ($Na_2B_4O_7 \cdot 10H_2O$), and 0.5 mM CTAB adjusted to pH 9.2. The separation buffer was filtered through a 0.20 μm membrane filter before use, and the standard procedure for capillary wash between analyses included a flush with 1.0 M NaOH for 2 min, water for 1 min, and separation buffer for 5 min. Separation was performed at −10 kV and 30 °C.

Relative Response Factors (RRF). The quantification of α-galactosides implied the use of RRF, which were determined relative to the internal standard maltitol from the slope (α) of the calibration curves for the α-galactosides and the internal standard according to the following equation: $RRF_x = \alpha_{maltitol}/\alpha_x$. The relative response factors determined was as follows: sucrose, 1.48; raffinose, 0.91; stachyose, 0.81; and verbascose, 0.94.

Statistics and Principal Component Analysis (PCA). Program GLM (SAS release 8.02 TS Level 02MO) was used for the variance analysis, and chemometric characterizations of the RFO data were performed by PCA using The Unscrambler v. 7.01 from Camo ASA, Oslo, Norway. Analyses were run of both the entire sample set containing *Lupinus*, *Brassica*, *Pisum*, and *Hordeum* samples as well as on *Lupinus* and *Brassica* samples individually.

RESULTS AND DISCUSSION

A newly developed separation method for RFOs based on indirect UV detection and free zone capillary electrophoresis (FZCE) of native α-galactosides (*23*) was used for this study. Sucrose and α-galactosides including raffinose, stachyose, verbascose, and ajugose were isolated from different varieties of *Brassica*, *Hordeum*, *Lupinus*, and *Pisum* species and analyzed by HPCE. An electropherogram showing separation of sucrose, raffinose, stachyose, verbascose, and ajugose from *Lupinus angustifolius* cv. LAG-24 (see **Table 1**) in the optimized HPCE system is shown in **Figure 1**. The results of the capillary electrophoresis are summarized in **Table 1**.

The *Brassica* varieties all contained the two lower homologues of RFO, raffinose and stachyose, as well as sucrose. In addition to this, the lupine samples also contained verbascose and ajugose. Sucrose, raffinose, stachyose, and verbascose were identified in the pea samples (*Pisum sativum* L. cv. Bodil), whereas only sucrose and raffinose were detected in the analyzed barley variety, *Hordeum vulgare* L. cv. Vega.

The average contents (grams per 100 g of seeds) of RFOs and total nonreducing sugars (sucrose + RFO) in the various genera and species were calculated from the data in **Table 1** and are summarized in **Table 2**. The largest amount of RFO was found in the lupine samples, in which 9.1 ± 2.6% w/w of

Table 1. Contents of Raffinose Family Oligosaccharides and Sucrose in Seeds of Various Varieties of *Lupinus*, *Pisum*, *Brassica*, and *Hordeum*[a]

genus	species	cv.	*n*	sucrose	raffinose	stachyose	verbascose	ajugose
Lupinus	*albus*	Amiga	3	67.0 BC (12.4)	17.2 B (15.7)	77.3 DE (10.9)	16.7 HI (7.4)	3.5 D (18.0)
		Feli	3	105.7 A (16.2)	11.5 DE (2.9)	102.6 B (9.4)	21.9 GH (18.4)	1.8 D (46.3)
		Minori	3	57.8 BCD (12.0)	13.8 BCD (13.1)	88.5 CD (10.6)	34.9 CD (7.4)	4.5 D (16.4)
		Nelly	3	92.5 A (8.6)	9.3 E (9.6)	94.0 BC (9.7)	13.4 I (10.6)	3.1 D (10.3)
		Portugal	3	63.5 BC (10.2)	11.4 DE (10.4)	66.8 E (12.7)	21.2 GH (2.5)	2.6 D (14.9)
		Weibit	3	60.6 BC (5.5)	13.1 CD (8.6)	77.2 DE (6.4)	22.7 FGH (6.5)	2.6 D (5.9)
	angustifolius	9909	3	56.3 CD (51.9)	10.4 DE (51.4)	37.5 FG (49.9)	21.4 GH (47.3)	18.6 C (47.8)
		E101	3	74.7 B (3.5)	13.9 BCD (6.0)	39.1 FG (5.6)	28.0 EFG (7.2)	18.3 C (5.2)
		LAG-24	3	94.2 A (4.3)	16.1 BC (1.8)	47.7 FG (0.8)	31.5 CDE (6.3)	16.7 C (7.5)
		Sonet	3	73.4 B (1.5)	11.9 DE (1.2)	36.3 G (1.8)	28.8 DEF (15.3)	21.0 BC (21.6)
		Zubra	3	50.7 CD (12.0)	16.7 BC (10.9)	49.8 F (11.1)	36.1 C (14.3)	26.2 B (14.7)
	luteus	Juno	3	28.8 E (6.8)	32.9 A (10.7)	12.7 H (9.8)	80.3 A (7.7)	68.7 A (7.6)
		Teo	3	28.4 E (6.5)	15.6 BC (3.4)	78.6 DE (5.4)	69.7 B (5.4)	6.6 D (17.6)
	mutabilis	91	3	40.7 DE (12.4)	29.5 A (8.7)	106.3 AB (7.1)	12.5 I (4.3)	2.2 D (25.6)
Pisum	*sativum*	Bodil	5	79.4 (8.4)	11.5 (6.6)	18.6 (7.6)	26.2 (10.2)	ND[b]
Brassica	*campestris*	Merit	2	131.8 BC (0.6)	2.3 J (3.7)	10.8 L (0.5)	ND	ND
	napus	Apex	2	130.4 BC (1.2)	5.2 EFG (2.2)	11.2 L (0.3)	ND	ND
		Canary	2	128.3 CD (1.0)	6.0 CD (2.3)	15.9 IJ (1.5)	ND	ND
		Capitol	2	126.9 CDE (0.3)	3.1 I (1.6)	23.2 B (0.3)	ND	ND
		Contact	2	111.0 H (4.5)	6.6 B (1.3)	12.7 K (0.8)	ND	ND
		Escort	2	132.2 BC (1.6)	6.4 BC (5.6)	18.6 EF (1.4)	ND	ND
		Express	2	116.1 GH (1.1)	2.7 IJ (3.9)	26.2 A (4.4)	ND	ND
		Fortress	2	124.1 DEF (3.3)	5.6 DE (0.6)	15.4 J (0.5)	ND	ND
		Gemini	2	134.3 B (0.8)	6.5 B (0.0)	19.2 DEF (0.6)	ND	ND
		Herald	2	141.2 A (1.3)	5.5 EFG (0.3)	18.2 FG (1.4)	ND	ND
		Lipton	2	121.1 FG (1.2)	5.1 FG (8.1)	17.1 GH (0.5)	ND	ND
		Lirajet	3	119.4 FG (4.1)	2.7 IJ (16.4)	21.5 C (7.1)	ND	ND
		Madrigal	2	122.8 EF (3.1)	6.6 B (1.7)	16.9 HI (1.6)	ND	ND
		Maskot	2	119.9 FG (0.8)	3.2 I (11.7)	11.7 KL (3.0)	ND	ND
		HEAR	3	89.7 J (1.9)	4.0 H (4.3)	14.9 J (1.9)	ND	ND
		Pollen	3	117.3 G (2.0)	3.1 I (10.5)	17.5 GH (4.0)	ND	ND
		Pronto	2	134.2 B (1.1)	5.0 G (2.7)	20.1 D (0.5)	ND	ND
		Synergy	2	130.0 BC (1.5)	5.4 EFG (1.1)	11.6 L (0.2)	ND	ND
		Tradition	2	101.7 I (1.4)	3.0 I (5.1)	15.1 J (1.2)	ND	ND
	nigra	nn[c]	3	74.7 K (2.6)	9.0 A (0.9)	19.4 DE (0.7)	ND	ND
Hordeum	*vulgare*	Vega	6	19.9 (6.7)	10.5 (4.5)	ND DE (0.7)	ND	ND

[a] Results are given as μmol/g. The names of the various varieties and the number of replicates are listed in the columns cv. and *n*, respectively. Two variance analyses were performed on the varieties within the genera *Lupinus* and *Brassica*, respectively. Values with insignificant difference ($P > 0.05$) are given the same letter marking within each of the two groups. Coefficient of variation (CV) is included in parentheses. [b] ND, not detected. [c] nn, unknown variety name.

Table 2. Average Content of Nonreducing Sugars in *Lupinus* and *Brassica* as well as in the Various Species of *Brassica* (*B. napus* L., *B. nigra* L., and *B. campestris* L.), *Hordeum* (*H. vulgare* L.), *Lupinus* (*L. albus* L., *L. angustifolius* L., *L. luteus* L., and *L. mutabilis* L.), and *Pisum* (*P. sativum* L.) Listed in **Table 1**[a]

genus	species	*n*	sucrose (g/100 g of seeds)	RFO (g/100 g of seeds)	total (g/100 g of seeds)
Lupinus		42	2.2 (37.9)	9.1 (28.5)	11.3 (21.1)
Pisum	*sativum*	5	2.7 (8.4)	4.0 (8.0)	6.7 (7.7)
Brassica		45	4.0 (15.2)	1.4 (32.8)	5.5 (11.8)
Hordeum	*vulgare*	6	0.7 (6.7)	0.5 (4.5)	1.2 (2.2)
Lupinus	*albus*	18	2.6 (27.0)	8.4 (14.4)	10.9 (14.7)
	angustifolius	15	2.4 (28.0)	7.9 (21.6)	10.3 (19.8)
	luteus	6	1.0 (6.0)	14.2 (14.9)	15.2 (14.1)
	mutabilis	3	1.4 (12.4)	9.8 (6.2)	11.2 (6.9)
Brassica	*campestris*	2	4.5 (0.6	0.8 (0.9)	5.3 (0.7)
	napus	40	4.1 (10.7)	1.4 (18.1)	5.5 (10.4)
	nigra	3	2.6 (2.6)	1.8 (0.7)	4.3 (1.3)

[a] The column "total" summarizes the average content of sucrose and raffinose family oligosaccharides (RFO). Coefficients of variation (CV) are indicated in parentheses.

the seeds on average derived from these types of α-galactosides. The *Brassica* samples contained 1.4 ± 0.5% RFOs (only raffinose and stachyose), whereas the barley sample, *H. vulgare* L. cv. Vega, contained 0.5% w/w raffinose, which was the only RFO component present in the *H. vulgare* seeds. The average contents of the individual RFOs and sucrose (micromoles per gram of seeds) in the *Lupinus*, *Pisum*, *Brassica*, and *Hordeum* genera and in the various species are summarized in **Table 3**.

The largest amounts of sucrose were detected in the *Brassica* species, which on average contained 121.1, 74.7, and 131.8 μmol/g, corresponding to 4.1, 2.6, and 4.5% w/w of the seed weight for *B. napus*, *B. nigra*, and *B. campestris*, respectively. In the lupine seed samples, the white (*L. albus*) and the blue lupine (*L. angustifolius*) accumulated the highest amount of sucrose (74.5 and 69.9 μmol/g, corresponding to 2.6 and 2.4% w/w of seed weight, respectively), whereas *L. luteus* and *L. mutabilis* contained 28.6 μmol/g (1.0% w/w) and 40.7 mol/g (1.4% w/w), respectively.

In all species analyzed the raffinose content was considerably lower than the sucrose content, averaging 15.9 μmol/g (1.0% w/w), 11.5 μmol/g (0.7% w/w), 4.9 μmol/g (0.3% w/w), and 10.5 μmol/g (0.6% w/w) in *Lupinus*, *Pisum*, *Brassica*, and *Hordeum*, respectively. The highest amount was detected in *L. mutabilis* L. cv. 91, containing 29.5 μmol/g of raffinose in the seed (1.8% w/w), whereas the raffinose content in the remaining *Lupinus* species ranged between 12.7 and 24.2 μmol/g of seed (0.8−1.6% w/w). *B. campestris* and *B. nigra* contained 2.3 and 9.0 μmol/g (0.1 and 0.5% w/w) of raffinose, respectively,

Table 3. Average Sucrose and Raffinose Family Oligosaccharide Content in *Brassica* and *Lupinus* as well as in the Various Species of *Brassica* (*B. napus* L., *B. nigra* L., and *B. campestris* L.), *Hordeum* (*H. vulgare* L.), *Lupinus* (*L. albus* L., *L. angustifolius* L., *L. luteus* L., and *L. mutabilis* L.), and *Pisum* (*P. sativum* L.) Listed in **Table 1**[a]

genus	species	*n*	sucrose (μmol/g of seed)	raffinose (μmol/g of seed)	stachyose (μmol/g of seed)	verbascose (μmol/g of seed)	ajugose (μmol/g of seed)
Lupinus		42	63.9 B (37.9)	15.9 A (43.7)	65.3 A (43.7)	31.4 A (63.0)	14.0 A (125.7)
Pisum	*sativum*	5	79.4 B (8.4)	11.5 AB (6.6)	18.6 B (7.6)	26.2 A (10.2)	
Brassica		45	118.5 A (14.6)	4.9 C (38.3)	17.0 B (23.7)		
Hordeum	*vulgare*	6	19.9 C (6.7)	10.5 B (4.5)			
Lupinus	*albus*	18	74.5 A (27.0)	12.7 B (22.4)	84.4 B (16.8)	21.8 C (32.8)	3.0 C (32.5)
	angustifolius	15	69.9 A (28.0)	13.8 B (23.9)	42.1 C (22.3)	29.2 B (23.5)	20.2 B (26.4)
	luteus	6	28.6 B (6.0)	24.2 A (40.2)	45.7 C (79.2)	75.0 A (9.9)	37.7 A (90.7)
	mutabilis	3	40.7 B (12.4)	29.5 A (8.7)	106.3 A (7.1)	12.5 D (4.3)	2.2 C (25.6)
Brassica	*campestris*	2	131.8 A (0.6)	2.3 B (3.7)	10.8 B (0.5)		
	napus	40	121.1 A (10.7)	4.6 B (31.3)	17.1 AB (23.2)		
	nigra	3	74.7 B (2.6)	9.0 A (0.9)	19.4 A (0.7)		

[a] Three variance analyses were performed on the four genera as well as on the species within *Lupinus* and *Brassica*, respectively. Values with insignificant difference ($P > 0.05$) are given the same letter within each of the three groups: genus (*Lupinus*, *Pisum*, *Brassica*, *Hordeum*), *Lupinus* (*L. albus*, *L. angustifolius*, *L. lutelus*, *L. mutabilis*), or *Brassica* (*B. napus*, *B. nigra*, *B. campestris*). Coefficients of variation (CV) are indicated in parentheses.

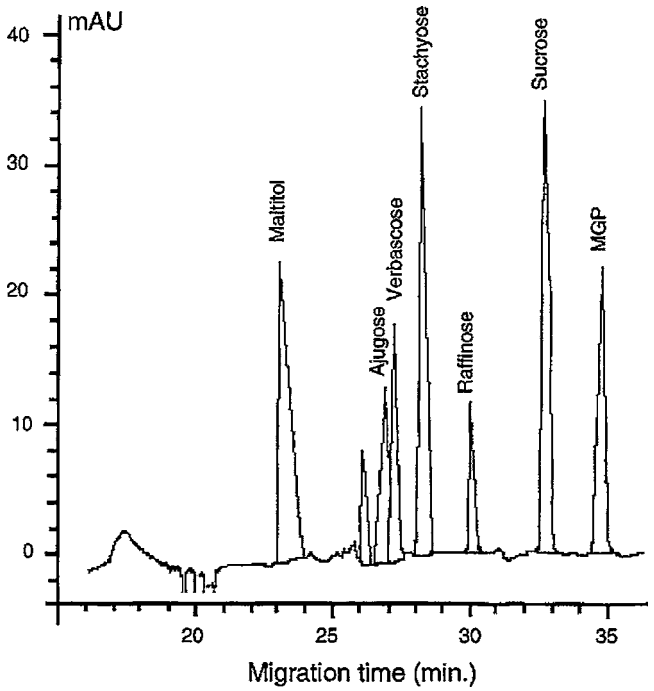

Figure 1. Electropherograms by HPCE with indirect UV detection of α-galactosides isolated from *L. angustifolius* (LAG-24; **Table 1**). Maltitol and MGP are both internal standards. Separation was performed at −10 kV and 30 °C in a separation buffer consisting of 50 mM disodium tetraborate, 0.5 mM cetyltrimethylammonium bromide, and 20 mM pyridine-2,6-dicarboxylic acid adjusted to pH 9.2. The signal wavelength was set at 350 nm with a reference at 275 nm.

whereas *B. napus* on average contained 4.6 μmol/g (0.3% w/w) raffinose in the seeds.

Large variations within the various species were seen in the stachyose content in the lupine samples with an average content of 65.3 ± 27.7 μmol/g of seeds. In the lupine samples stachyose was the most abundant RFO, which is in agreement with the findings of Horbowicz and Oberndorf (*27*). The lowest amount of stachyose was found in *L. angustifolius* (42.1 μmol/g; 2.8% w/w) followed by *L. luteus*, *L. albus*, and, with the highest content, *L. mutabilis*, containing 45.7, 84.4, and 106.3 μmol/g, equivalent to 3.0, 5.6, and 7.1% w/w of seed weight, respectively. In the *Brassica* species the stachyose content was considerably lower, ranging from 10.8 to 19.4 μmol/g, corresponding to 0.7−1.3% w/w of the seed weight. The pea sample (*P. sativum*) contained 18.6 μmol of stachyose/g of seed, and in the barley sample no stachyose was detected.

The verbascose content occurring in the *Lupinus* and *Pisum* samples was found in the range from 12.5 μmol/g (1.0% w/w) in *L. mutabilis* to 75.0 μmol/g (6.2% w/w) in the seeds of *L. luteus*.

The electropherogram in **Figure 1** shows that minor components migrate just before the ajugose peak at 26.96 min. These unknown compounds, which might be higher homologues of the RFO series, sometimes interfere with the ajugose peak and decrease the reliability of the ajugose quantification. For this reason the ajugose data should be regarded as estimates.

The average ajugose content in the analyzed lupine samples was 14.0 μmol/g (1.4% w/w) (**Table 3**), where *L. luteus* has an estimated ajugose content of 37.7 μmol/g (3.7% w/w). In the *L. mutabilis*, *L. albus*, and *L. angustifolius* species the ajugose contents were 2.2, 3.0, and 20.2 μmol/g of seed (0.2, 0.3, and 2.0% w/w), respectively.

Chemometric tools were used for graphical plots of the variations in the measured data matrix. Chemometrics can be defined as optimal extraction of the most important information from measured data using the requisite mathematics and statistics, which in this case are provided by The Unscrambler from CAMO. The PCA used in the present study is generally used for explorative data structure modeling by evaluating data in a graphical context, to investigate for patterns and relationships between different parameters, in this case RFOs.

By matrix notation the principles of PCA can be expressed as $\boldsymbol{X} = \boldsymbol{T}\cdot\boldsymbol{P}^{\boldsymbol{T}} + \boldsymbol{E}$, where $\boldsymbol{X}$ is the variable matrix, $\boldsymbol{T}$ is a matrix of *score values*, $\boldsymbol{P}^{\boldsymbol{T}}$ is a transposed matrix of *loading values*, and $\boldsymbol{E}$ is a matrix containing the residual variance. In this way the $\boldsymbol{X}$ matrix is decomposed into a structural part consisting of $\boldsymbol{T}\cdot\boldsymbol{P}^{\boldsymbol{T}}$ and the $\boldsymbol{E}$ matrix describing the noise. The loading matrix $\boldsymbol{P}$ consists of orthogonal vectors decreasingly describing the direction of the sample variation. The score matrix vectors relate the sample values to the coordinate system described by the loading matrix. Thus, the first principal component (PC1) provides information about the direction in the data space that describes the largest variation, PC2 gives the direction of the next largest variation, and so on.

All RFO data from the samples in **Table 1** were included in a PCA from which score and loading plots of PC1 versus PC2 are shown in **Figure 2**. Just below the score and loading plots

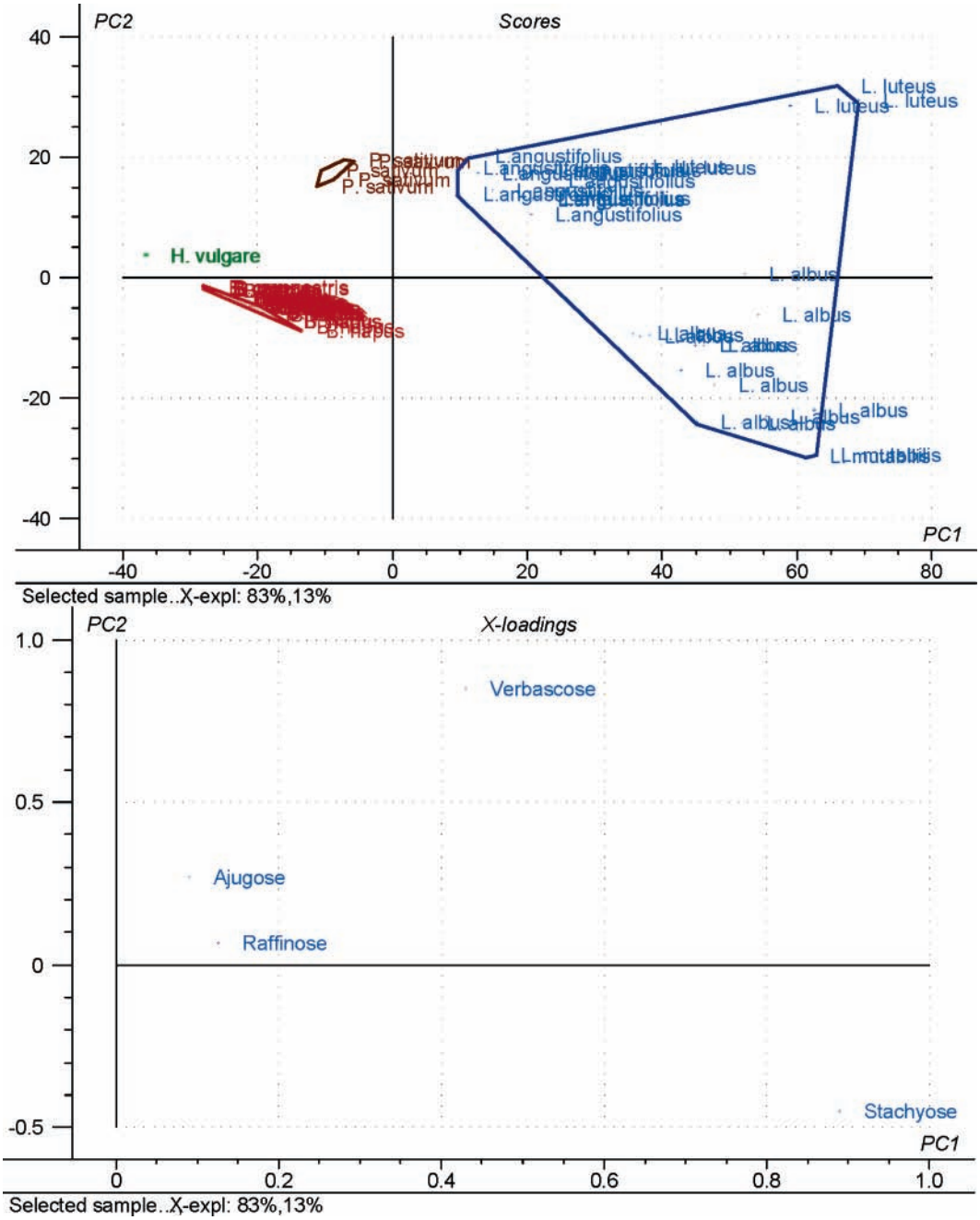

Figure 2. Chemometric analyses of centered raffinose, stachyose, verbascose, and ajugose data from various *Brassica*, *Lupinus*, *Pisum*, and *Hordeum* varieties: (top) score plot of PC1 versus PC2; (bottom) loading plot; red, *Brassica*; blue, *Lupinus*; brown, *Pisum*; green, *Hordeum* (see **Table 1**).

are indicated the part of the sample variation, which each of the individual principal components describes. In this case these plots describe 96% of the RFO variation, as 83% are described by PC1 and 13% of the variation is accounted for by PC2. At the loading plot at the left side of the figure, the influences of the individual parameters on the plotted principal components (in this case PC1 and PC2) are shown. PC1 mainly describes variations in stachyose contents, whereas the PC2 direction almost entirely relates to verbascose content variations. Verbascose has relatively great influence on both PC1 and PC2, which results in increased score values in both directions when samples contain much verbascose. Stachyose has a negative influence on PC2, meaning that large amounts of stachyose decrease PC2 score values. It is further seen that raffinose and ajugose only have limited influence on both PC1 and PC2.

At the score plot the genera could be separated into four groups. *Hordeum*, having raffinose as the only RFO present in the seeds, showed large negative PC1 score values. Because the various RFO parameters were centered during PCA, placing the origin of the principal component coordinate system in the center of the data space, this indicates below-average contents of stachyose and verbascose. Both raffinose and stachyose are present in the *Brassica* samples, resulting in higher PC1 score values than in the *Hordeum* samples, although still negative due to the relatively low content of these RFOs. The PC2 score values were negative as the influence of stachyose in the negative PC2 direction overrules the minor positive contribution from the raffinose contents. The *Pisum* samples showed small negative PC1 but large positive PC2 score values due to relatively limited stachyose content compared to the high amount of verbascose present in the pea seeds. All of the *Lupinus* samples had positive PC1 score values due to the presence of high amounts of both stachyose and verbascose. Group formations within the lupine samples could be seen at the score plot,

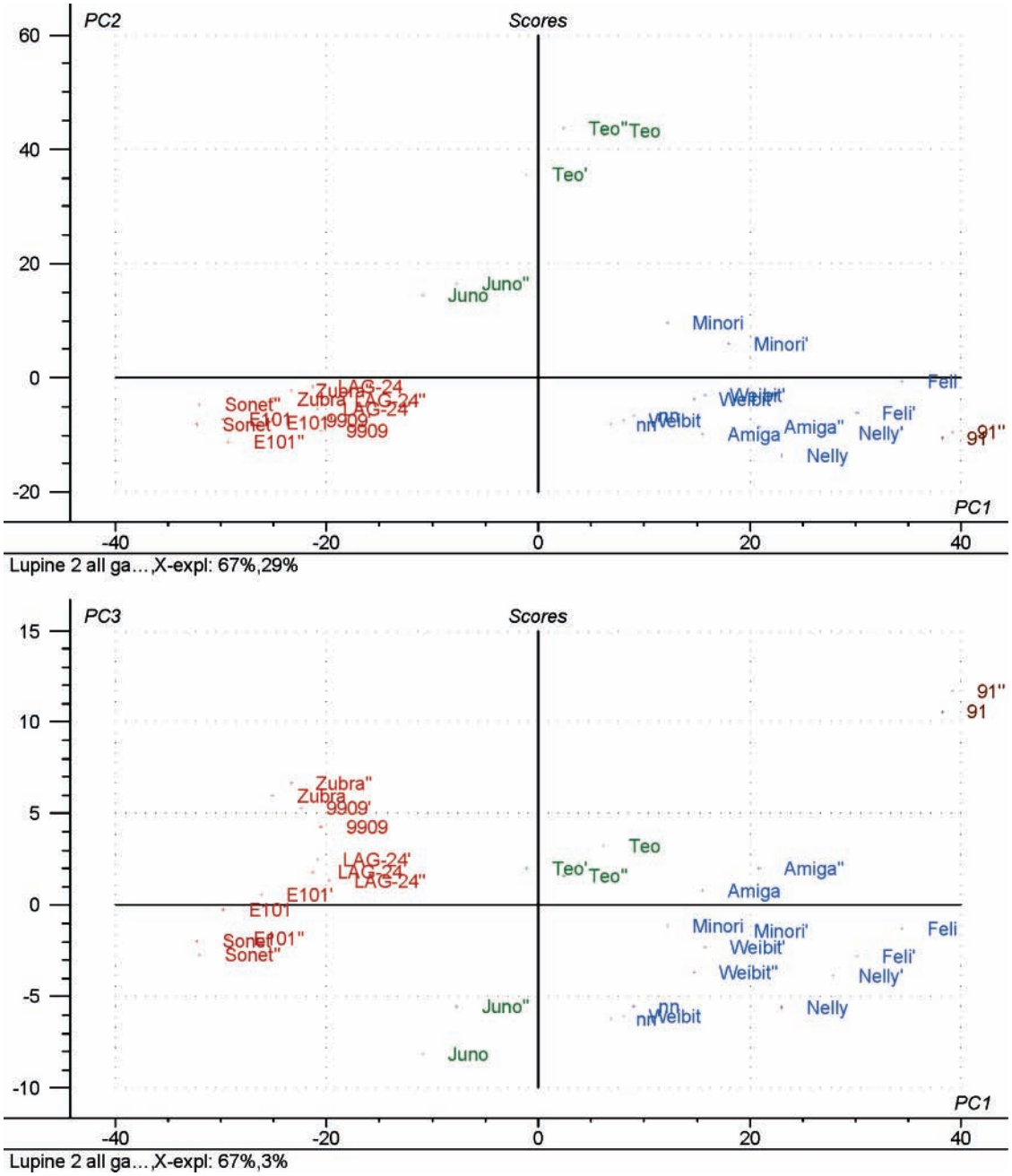

Figure 3. Score plots of chemometric analyses of *Lupinus* samples from **Table 1** using centered raffinose, stachyose, verbascose, and ajugose data: (top) PC1 versus PC2; (bottom) PC1 versus PC3; blue, *L. albus*; red, *L. angustifolius*; green, *L. luteus*; brown, *L. mutabilis*. The corresponding loading plots are shown in **Figure 4**.

and for the *Lupinus* samples RFO variation varies as function of the *Lupinus* species.

New PCA using the lupine sample RFOs alone resulted in score and loading plots shown in **Figures 3** and **4**, respectively (PC1 versus PC2 at the top and PC1 versus PC3 at the bottom). In this case 99% of the lupine sample RFO variation was described by PC1, PC2, and PC3 (67, 29, and 3%, respectively). The loading plots showed that positive PC1 values indicate high stachyose and low ajugose contents, whereas negative PC1 values indicate high ajugose and low stachyose contents. High verbascose content resulted in high PC2 score values, whereas high PC3 score values derived from high raffinose or ajugose contents. The score plots showed high contents of stachyose and low contents of ajugose in the *L. albus* samples, in contrast to *L. angustifolius* having negative PC1 score values indicating low stachyose and high ajugose contents. The *L. luteus* samples had average stachyose and ajugose contents but large PC2 score values due to large amounts of verbascose in the seeds. The *L. mutabilis* had very high score values in the PC1 direction and average PC2 score values, resulting in placement at the PC1 versus PC2 score plot near the *L. albus* samples. From the PC1 versus PC3 plot it can be seen that the high raffinose contents clearly separates *L. mutabilis* from *L. albus*.

In the *Brassica* samples only raffinose and stachyose were measured by the RFO analyses. When PCA was applied to these samples, the PC1 related entirely to variations in the stachyose contents and accounted for 82% of the total sample variation (**Figure 5**). The remaining 18% of the variation was described by PC2 and related to the raffinose variation. *B. nigra* L. cv. nn showed an above-average stachyose content and clearly higher raffinose content compared to the other *Brassica* species. *B. campestris* L. cv. Merit had, on the other hand, the lowest

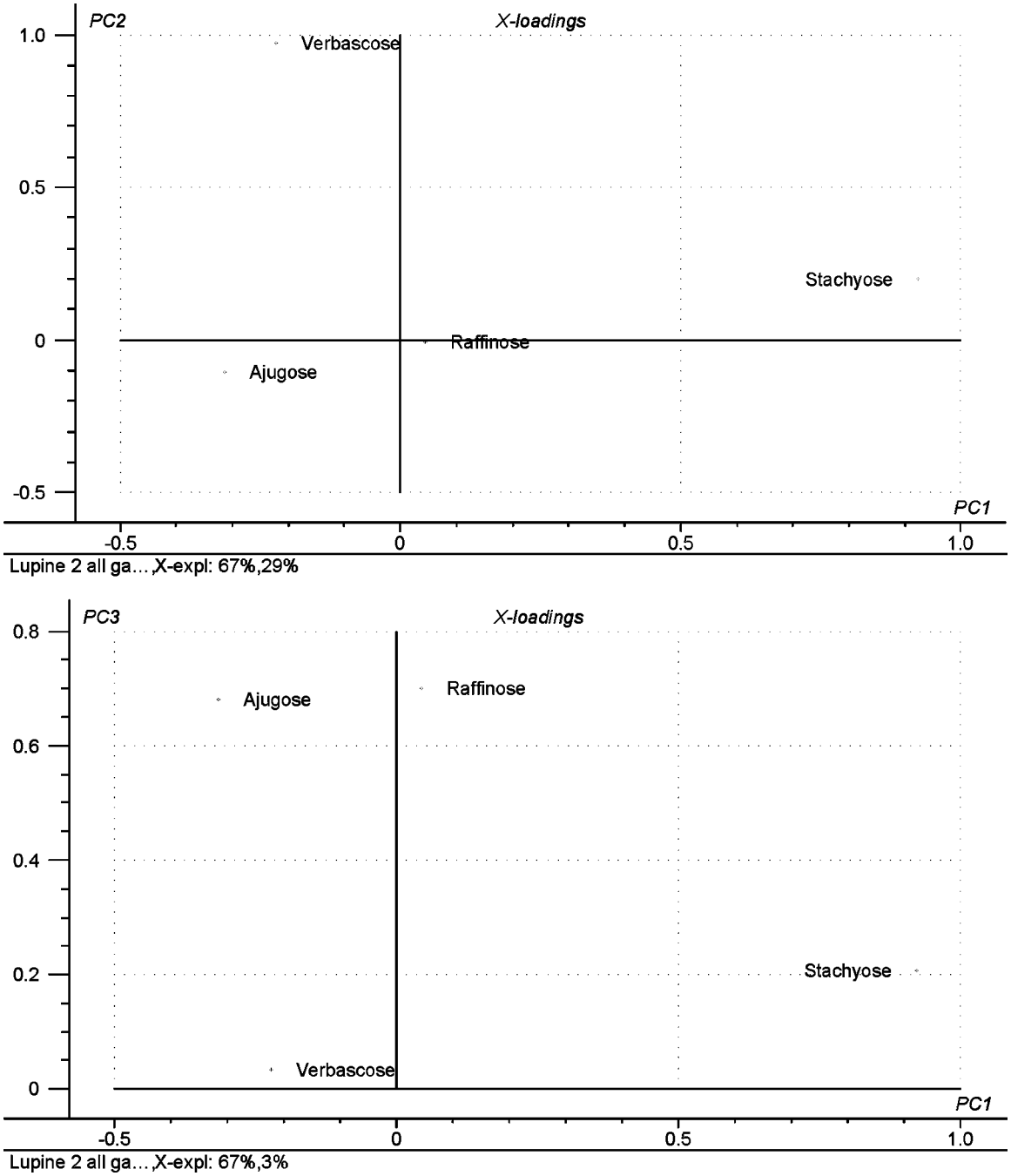

Figure 4. Loading plots of chemometric analyses of *Lupinus* samples. For details see **Figure 3**.

amounts of both raffinose and stachyose present in the seeds and is thus placed lowest to the left in the PC1 versus PC2 score plot. The *B. napus* samples were placed around the center of the plot, although with a tendency to the formation of five subgroups. The Mascot variety was located near the *B. campestris* sample (cv. Merit), thus having low amounts of both RFOs. HEAR, Pollen, and Tradition cultivars had low raffinose contents but an average stachyose content, whereas the Capitol, Express, and Lirajet cultivars had low raffinose and very high stachyose contents. The remaining varieties showed average or above-average raffinose contents in addition to a low stachyose content for Apex, Contact, and Synergy and average or above-average stachyose contents for Canary, Escort, Fortress, Gemini, Herald, Lipton, Madrigal, and Pronto.

The PCA clearly showed that the RFO composition in the seeds depends on the plant genera, but especially for *Lupinus* and to some extent *Brassica*, the RFO data indicated a tendency for RFO composition to depend on the specific species as well. A recent study on pea seeds using high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) has indicated similar patterns in this Leguminosae genus (*8*).

The examined material was not sufficient for preparing a model for the prediction of plant genus and/or species based on RFO data. However, PCA makes it possible to graphically identify patterns within the various samples. Factors such as environmental stress, degree of ripeness, and germination influence the content of α-galactosides in seeds and germinating seedlings (*28*−*30*). Thereby, it may also have an influence on the nutritional values (*22*), even as several compounds in the complete matrix systems used as feed and food define the quality or nutritional value (*3*, *12*−*15*, *17*−*19*). Recent research has also focused on the effects from carbohydrates and dietary fibers in relation to functional properties and benefits when used in

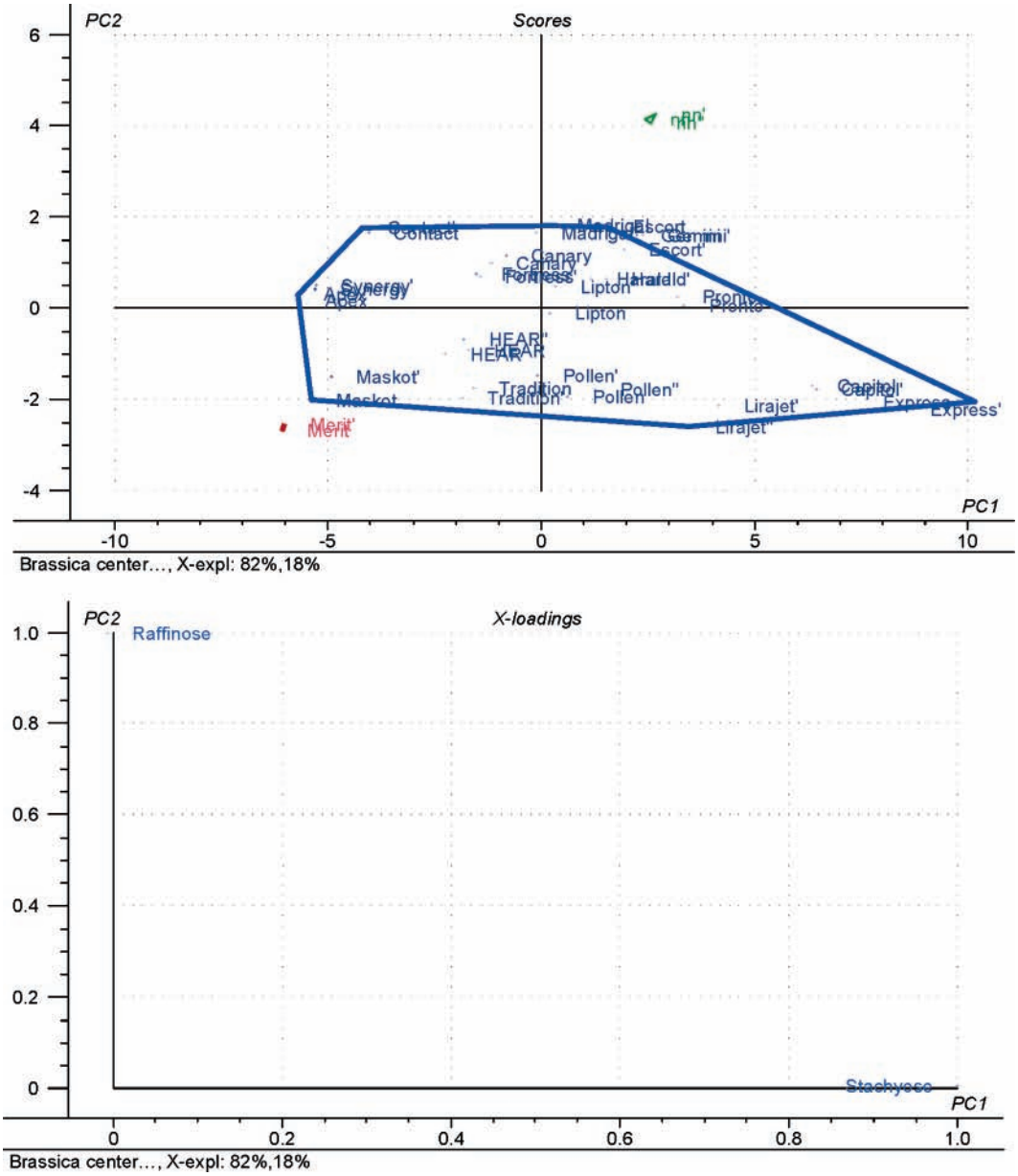

Figure 5. Chemometric analyses of *Brassica* samples from **Table 1** using centered raffinose and stachyose data: red, *B. campestris*; blue, *B. napus*; green, *B. nigra*. Score and loading plots as in **Figure 2**.

human nutrition (*4*, *6*, *7*, *9*). More specific information on physicochemical−biochemical properties of structurally different oligosaccharides or mixtures thereof is needed, which seems to be possible to obtain by use of information now available (*8*, *10*, *11*) and given by the present work. A larger data set will, thus, make the prediction of plant origin possible when unknown RFO-containing materials are analyzed, even though the above-mentioned factors must be taken into account when methods for the prediction of RFO origin are developed.

ABBREVIATIONS USED

B., *Brassica*; CTAB, cetyltrimethylammonium bromide; CV, coefficient of variation; cv., cultivar; FZCE, free zone capillary electrophoresis; *H.*, *Hordeum*; *L.*, *Lupinus*; LMW, low molecular weight; MGP, methyl glucopyranoside; nn, no name (unknown cultivar); *P.*, *Pisum*; PC, principal component; PCA, principal component analysis; PDC, pyridine-2,6-dicarboxylic acid; RFO, raffinose family oligosaccharides; RRF, relative response factor; SCFA, short-chain fatty acids.

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Received for review November 30, 2004. Revised manuscript received April 15, 2005. Accepted April 18, 2005. The Commission of the European Union (FOOD-CT-2004-506-223 and QLK1-CT-2002-02235) is gratefully acknowledged for financial support of this work.

JF040471V