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Selecting Sprouts of Brassicaceae for Optimum Phytochemical Composition

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ABSTRACT: Cruciferous foods (Brassicaceae spp.) are rich in nutrients and bioactive compounds. Edible sprouts are becoming popular fresh foods and, therefore, the phytochemical profiling of nine varieties of Brassicaceae (broccoli, kohlrabi, red cabbage, rutabaga, turnip, turnip greens, radish, garden cress, and white mustard) was evaluated for this purpose. The glucosinolates in seeds were significantly higher than in sprouts, and day 8 of germination was considered the optimum for consumption. The sprouts with higher concentrations of glucosinolates in 8-day-old sprouts were white mustard, turnip, and kohlrabi (~815, ~766, and ~653 mg 100 g^{-1} FW, respectively). Red cabbage and radish presented great total glucosinolates content (~516 and ~297 mg 100 g^{-1} FW, respectively, in 8-day-old sprouts) and also higher total phenolic contents, biomass, and antioxidant capacity. The selection of the best performers in terms of germination quality and phytochemical composition is the key to optimize new fresh foods enriched in health-bioactive compounds. Further research on the bioavailability of the bioactive compounds in *Brassica* foods will allow backing of recommendations for dietarily effective dosages for nutrition and health.

KEYWORDS: *germination, seeds, glucosinolates, phenolics, biomass, HPLC-PDA-ESI-MSn*

INTRODUCTION

Brassicaceae vegetables, or cruciferous foods, include a variety of horticultural crops with global economical relevance (oilseeds, forage, condiments, and vegetables). In Spain (Murcia), broccoli and cabbage (>190,000 tons) are a major agroeconomical activity.[1] Genomics studies of the U triangle[2] showed that *Brassica oleracea* (such as kale, cabbage, broccoli, and kohlrabi), *Brassica rapa* (such as turnip and Chinese cabbage), and *Brassica nigra* (black mustard) all originated from a common ancestral. Other species from this family are *Brassica napus* (such a rutabaga, rapeseed, and nabicol), *Raphanus sativus* (radish), *Lepidium sativum* (garden cress), and *Sinapis alba* (white mustard). *Brassica* vegetables have received considerable research attention because of their association with health-promoting effects including improving the immune system, protection against allergies, antihypertensive properties, and reducing the risk for cardiovascular diseases and certain types of cancer.[3−5] Even if these vegetables are mainly recognized for their nitrogen−sulfur compounds, the glucosinolates, Brassicaceae foods are also rich in phenolic compounds, vitamins (A, C, E, and K), and minerals.[6] The content of bioactive compounds in Brassicaceae vegetables varies with genotype,[7,8] environmental stress,[9] growth conditions,[10] and storage, processing, and cooking methods.[11,12] Phenolic compounds and glucosinolates are present in high amounts in seeds and during the first days of germination, reaching a 10-fold increase compared to commercial adult plants.[13] Glucosinolates, nitrogen−sulfur compounds (β-D-thioglucoside-*n*-hydroxysulfates), are classified as aliphatic (the major group in almost all crucifer seeds and sprouts of *B. oleraceae*, *B. napus*, *B. rapa*, and *R. sativus*), indolic (representing lower amounts in the glucosinolate profile), or aromatic (characteristic in *S. alba* and *L. sativum*.[14,15]) and have been extensively studied due to their hydrolysis compounds, the isothiocyanates (such as sulphoraphane[16] and benzyl isothiocyanate[17]) and indoles (indol-3-carbinol), which are associated with a reduced risk for particularly cancers of the gastrointestinal tract, lung, and prostate. In contrast, progoitrin, also present in crucifers, is an "undesirable" glucosinolate, because it is converted to the antithyroid goitrin after myrosinase hydrolysis.[18] The phenolic profile of sprouts is composed mostly of sinapic acid derivatives (hydroxycinnamic acids), a small portion of flavonoids (mainly quercetin and kaempferol commonly found as *O*-glycosides, and also isorhamnetin, characteristic of *B. rapa* species), and other hydroxycinnamic acids (chlorogenic, *p*-coumaric, and ferulic acids and their derivatives).[19,20] Brassicaceae sprouts are becoming popular health-food items and widely recommended by dieticians (highly nutritious, low-fat foods, rich in health-promoting phytochemicals, safe, and fresh); likewise, consumers are demanding foods to enjoy and promote wellness.[14]

The aim of the present work was to characterize nine varieties of Brassicaceae, highlighting their glucosinolate contents and natural antioxidants (phenolic compounds and in vitro antioxidant capacity) to foster their applications as naturally healthy foods.

MATERIALS AND METHODS

Plant Material and Experimental Conditions. Seeds provided by Intersemillas S.A. (Valencia, Spain) were of commercial quality of ready-for-sprouting lines. Nine varieties were used: broccoli (*B. oleracea* L. var. *italica*), kohlrabi (*B. oleracea* L. var. *gongylodes*), red cabbage (*B. oleracea* L. var. *capitata*), rutabaga (*B. napus* L. var. *napobrassica*), turnip greens ('Globo Blanco', WAM seeds, Galicia) and turnips (*B. rapa* L. subsp. *rapa*), radish (*R. sativus*), garden cress (*L. sativum*), and white mustard (*S. alba*). Seeds were rinsed in

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Table 1. Data of Biomass Increase Ratio (Sprouts vs Seeds) in Brassicaceae Sprouts[a]

variety	scientific name	% germination	day 4	day 8	day 12	ANOVA[b]	LSD $_{0.05}$[c]
broccoli	*Brassica oleracea* var. *italica*	>90	2.18a	3.17a	3.33a	ns	0.75
kohlrabi	*Brassica oleracea* var. *gongylodes*	7	0.28cdB	0.70cA	0.97bcA	**	0.12
red cabbage	*Brassica oleracea* var. *capitata*	5	0.79bcB	1.03bcB	1.90abA	***	0.11
rutabaga	*Brassica napus* var. *napobrassica*	8	1.21bB	2.73aAB	3.33aA	*	0.51
turnip green	*Brassica rapa* var. *rapa*	10	0.88bB	2.23abA	3.47aA	**	0.42
turnip	*Brassica rapa* var. *rapa*	2	0.29 cd	0.57c	0.87bc	ns	0.22
radish	*Raphanus sativus*	17	1.27bB	2.77aA	2.83aA	*	0.46
garden cress	*Lepidium sativum*	49	0.00dB	0.60cA	0.60cA	***	0.05
white mustard	*Sinapis alba*	18	0.32cdC	1.40bcB	2.37aA	***	0.14
LSD$_{0.05}$[c] (ANOVA $p < 0.001$)			0.15	0.37	0.25		

[a]Mean values ($n = 3$). Different lower case letters indicate statistically significant differences among varieties (for each sampling day). Different upper case letters indicate statistically significant differences between days (for each variety). [b]Levels of significance for each sampling day between species. Nonsignificant at $p > 0.05$ (ns); significant at $p < 0.05$ (*); significant at $p < 0.01$ (**); significant at $p < 0.001$ (***). [c]Least significant difference (LSD) for separating means in the respective column. The LSD was computed only after analysis of variance indicated a significant ($p < 0.05$) entry effect.

distilled water, immersed in 5 g L^{-1} sodium hypochlorite for 2 h, and drained and placed in distilled water under aeration overnight. After the soaking water had been poured off, the seeds were weighed (day 0) and spreaded evenly on trays (5 g per tray) lined with cellulose growth pad (CN Seeds, UK) and irrigated with Milli-Q water. Aliquots of 5 g of seeds were frozen in liquid nitrogen and stored at −80 °C pending phytochemical analysis.

The trays were transferred to a controlled environment chamber with a 16 h light/8 h dark cycle and air temperatures of 25 and 20 °C, respectively. The relative humidity (RH) was 60% (day) and 80% (night). Photosynthetically active radiation (PAR) of 400 μmol m^{-2} s^{-1} was provided by a combination of fluorescent tubes (Philips TLD 36 W/83, Hamburg, Germany; Sylvania F36W/GRO, Danvers, MA, USA) and metal halide lamps (Osram HQI.T 400 W, Munich, Germany). Brassicaceae sprouts were allowed to grow until they reached 12 days of age. Sprout samples (all sprouts from a single tray, germinated from 5 g of seeds) were collected at different time points after germination (days 4, 8, and 12). Three subsamples were rapidly and gently collected, always at 10 a.m., in the middle of the light period, taking three replicates for analysis. All samples were weighed (fresh mass), collected separately, flash frozen in liquid nitrogen, and stored at −80 °C prior to analyses.

Antioxidant Capacity Assay. The free radical-scavenging activity was determined using the free radical DPPH$^{\bullet}$ as well as the ferric reducing antioxidant power (FRAP) assay in aqueous media according to the procedure of Mena et al.[21] Freeze-dried fine powdered samples (100 mg) were extracted with 10 mL of MeOH for 60 min in an ultrasonic bath (5510E-MTH, Danbury, CT, USA) and then were centrifuged at 10480*g* (model EBA 21, Hettich Zentrifugen) during 15 min at room temperature. Results were expressed as millimolar Trolox equivalents (TE) per 100 g FW.

Extraction and Determination of Glucosinolates and Phenolic Compounds. *Sample Extraction.* Freeze-dried samples (100 mg) were extracted with 1.5 mL of 700 g L^{-1} methanol in a sonicator bath for 10 min, then heated at 70 °C for 30 min in a heating bath, with shaking every 5 min using a vortex stirrer, and centrifuged (17500*g*, 30 min, 4 °C). The supernatants were collected, and methanol was completely removed using a rotary evaporator. The dry material obtained was dissolved in 1 mL of ultrapure water and filtered through a 0.45 μm Millex-HV13 membrane (Millipore Corp., Bedford, MA, USA). Freeze-dried powder samples (1g) were homogenized three times with 25 mL of 700 g L^{-1} methanol. The homogenates were filtered through cheesecloth and kept in ice. The homogenates were subsequently centrifuged (3600*g*, 5 min, 4 °C), and the supernatants were evaporated under vacuum at 30 °C to approximately 1 mL, diluted to 2 mL with water, and filtered through a 0.45 μm Millex-HV13 membrane (Millipore Corp.). Caffeoylquinic acid derivatives were quantified as chlorogenic acid (5-caffeoylquinic acid; Sigma, St. Louis, MO, USA), flavonoids as quercetin 3-rutinoside (Sigma), and sinapic acid and ferulic derivatives as sinapic acid (Sigma). The total analyte content of phenolic compounds in broccoli sprouts was expressed as milligrams per 100 g FW.

HPLC-PDA-ESI-MSn Qualitative and Quantitative Analysis of Glucosinolates and Phenolic Compounds. Glucosinolates and phenolic compounds were determined using a LC multipurpose method that simultaneously separates intact glucosinolates and phenolics, according to the procedure of Francisco et al.,[19] with slight modifications. The separated intact glucosinolates, hydroxycinnamic acids (chlorogenic acid derivatives and sinapic acid derivatives), and flavonols were identified following their MS2$[M - H]^-$ fragmentations (and also MS3 fragmentation of the major MS2 ions for hydroxycinnamic acids and flavonols), UV−visible spectra, and the order of elution previously described for similar acquisition conditions.[19,22]

Glucosinolates were quantified in the HPLC-PDA using sinigrin as standard (sinigrin monohydrate; Phytoplan Diehm & Neuberger, GmbH, Heidelberg, Germany). Caffeoylquinic acid derivatives were quantified as chlorogenic acid (5-caffeolylquinic acid, Sigma-Aldrich Chemie GmbH, Steinheim, Germany), flavonols (quercetin and kaempferol derivatives) as quercetin-3-rutinoside (Merck, Darmstadt, Germany), and sinapic acid derivatives as sinapic acid (Sigma).

Statistical Methods. All assays were conducted in triplicate. The data were processed using the SPSS 17.0 software package (LEAD Technologies, Inc., Chicago, IL, USA). A Student's *t* test was used to determine the significance of differences between means. A multifactorial analysis of variance (ANOVA) and Tukey's multiple-range test were carried out to determine significant differences at *p* values <0.05. Pearson correlation analyses were also performed to corroborate relationships between selected parameters.

RESULTS AND DISCUSSION

Biomass. The seeds used in the experiments were obtained of commercial quality for sprouting; therefore, the germination rate is usually lower than in the varieties used for plant production. Only broccoli seeds reached >90% of germination (Table 1), whereas garden cress seeds germinated 50%, and the rate was <20% for the remaining seeds of the different varieties (Table 1). Table 1 shows an increasing biomass ratio from day 0 to days 4, 8, and 12. Broccoli sprouts showed the highest values, increasing 2-fold at day 4 and 3-fold at day 12 and exhibiting the highest percentage of germination. The 8- and 12-day-old sprouts were more desirable for consumption and marketing than the 4-day-old ones (not ready for manipulation). At day 8 of the monitored period, broccoli, rutabaga, turnip greens, and radish had biomass ratios significantly higher than the rest (2−3-fold), consistent with the greater length

Table 2. Glucosinolates Detected and Presented in Brassicaceae Seeds and Sprouts[a]

peak	code	compound	glucosinolate (gls) semisystematic name	class	t_R (min)	$[M - H]^-$ (m/z)	broccoli	kohlrabi	red cabbage	rutabaga	turnip greens	turnip	radish	garden cress	white mustard
1	GIB	glucoiberin	3-methylsulfinylpropyl-gls	aliphatic	6.5	422	+	+	+	0	0	0	0	+	+
2	PRO	progoitrin	(*R*)-2-hydroxy-3-butenyl-gls	aliphatic	7.1	388	+	+	+	+	+	+	0	+	0
3	GRE	glucoraphenin	4-methylsulfinyl-3-butenyl-gls	aliphatic	7.1	434	0	0	0	0	0	0	+	+	0
4	GRA	glucoraphanin	4-methylsulfinylbutyl-gls	aliphatic	7.2	436	+	+	+	+	+	+	0	+	+
5	EPRO	epiprogoitrin	(*S*)-2-hydroxy-3-butenyl-gls	aliphatic	8.0	388	0	0	+	0	0	0	0	0	+
6	SIN	sinigrin	2-propenyl-gls	aliphatic	8.4	358	+	+	+	+	+	+	0	+	+
7	GAL	glucoalyssin	5-methylsulfinylpentyl-gls	aliphatic	12.7	450	+	+	+	+	+	+	+	+	0
8	GSI	glucosinalbin	4-hydroxybenzyl-gls	aromatic	13.6	424	+	+	0	0	+	0	0	+	+
9	GNL	gluconapoleiferin	(*R*)-2-hydroxy-4-pentenyl-gls	aliphatic	14.3	402	0	0	+	+	+	+	0	+	0
10	GNA	gluconapin	3-butenyl-gls	aliphatic	17.5	372	+	+	+	+	+	+	+	+	+
11	GIV	glucoiberverin	3-methylthiopropyl-gls	aliphatic	19.5	406	+	+	0	0	0	0	+	0	0
12	OHGBS	4-hydroxyglucobrassicin	4-hydroxy-3-indolylmethyl-gls	indolic	20.0	463	+	+	+	+	+	+	+	+	+
13		*n*-butyl	*n*-butyl-gls	aliphatic	20.9	374	0	0	+	+	+	+	0	0	0
14	GTP	glucotropaeolin	benzyl-gls	aromatic	20.9	408	0	0	0	0	0	0	0	+	+
15	GBN	glucobrassicanapin	4-pentenyl-gls	aliphatic	22.7	386	0	0	+	+	+	+	0	0	0
16	GER	glucoerucin	4-methylthiobutyl-gls	aliphatic	23.7	420	+	+	0	0	0	0	0	0	0
17	DER	dehydoerucin	4-methylthio-3-butenyl-gls	aliphatic	24.9	418	0	0	0	+	0	0	+	0	0
18		*n*-pentyl	*n*-pentyl-gls	aliphatic	26.0	388	+	+	+	0	+	+	0	0	0
19	GBS	glucobrassicin	3-indolylmethyl-gls	indolic	26.4	447	+	+	+	+	+	+	+	+	+
20	GBT	glucoberteroin	5-methylthiopentyl-gls	aliphatic	28.2	434	+	+	0	+	0	0	+	+	+
21	GST	gluconasturtin	2-phenylethyl-gls	aromatic	28.3	422	+	+	+	+	+	+	0	+	+
22	MGBS	4-methoxyglucobrassicin	4-methoxy-3-indolylmethyl-gls	indolic	28.6	477	+	+	+	+	+	+	+	+	+
23		*n*-hexyl	*n*-hexyl-gls	indolic	31.4	402	+	+	+	+	+	+	0	0	0
24	NGBS	neoglucobrassicin	*N*-methoxy-3-indolylmethyl-gls	indolic	32.5	477	+	+	+	+	+	+	0	+	+

[a]Identification based on $[M - H]^-$ (m/z), retention time (t_R), and characteristic spectra. +, compound presence; 0, compound absence.

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(between 4 and 5 cm) (Table 1). On day 12, in addition to the above, red cabbage and white mustard reached significantly higher biomass values (2−3-fold) and greater growth (between 5 and 6 cm length). The higher values of biomass are indicative of better sprout growth (length) and better rate of fresh weight (FW) production. The biomass data of sprouts is not widely available in the literature. Gu et al.,[23] for example, recorded that broccoli sprouts grew rapidly after 36 h of germination, and similar sprout length as in our study was reported.[24] The early, noninvasive, and direct parameter of biomass is therefore a useful parameter to screen plant material for production of sprouts.

Glucosinolates. The main characteristic of the Brassicaceae wellness composition is their glucosinolate (GLS) profile;[8,25,26] therefore, the presence of individual intact GLSs was studid in seeds and sprouts (Table 2). The molecular ion $[M - H]^-$ (m/z) of GLSs, their fragmentation ion patterns, and retention times allowed the identification of 24 different compounds. The MS2 fragmentation of aglycone side chain produces the most consistent ion at m/z 259, and the MS3 fragmentation of this ion gives rise to fragments at m/z 97 (corresponding to the sulfate group) by the disassociation of GLSs in the ion trap mass spectrometer, constituting a very useful preliminary screening method for determining the presence of GLS in sprouts extracts.[22] Results showed significant differences of the characteristic GLS profiles among samples. All of the varieties studied contained common GLSs: gluconapin (**10**), 4-hydroxiglucobrassicin (**12**), glucobrassicin (**19**), 4-methoxyglucobrassicin (**22**), gluconasturtin (**21**), neoglucobrassicin (**24**), and glucoraphanin (**4**), except for radish, which did not contain the last three compounds. In *B. oleracea* species, kohlrabi and broccoli, we found identical GLS profiles [glucoiberin (**1**), progoitrin (**2**), **4**, sinigrin (**6**), glucoalyssin (**7**), glucosinalbin (**8**), **10**, glucoiberverin (**11**), **12**, glucoerucin (**16**), *n*-pentyl (**18**), **19**, glucoberteorin (**20**), **21**, **22**, *n*-hexyl-gls (**23**), and **24**]. By contrast, the red cabbage samples showed certain differences having epiprogoitrin (**5**), gluconapoleiferin (**9**), *n*-butyl-gls (**13**), and glucobrassicanapin (**15**), and not presenting compounds **7**, **11**, **16**, and **20**, being closely related to rutabaga, which differed in only five GLSs (containing **17** and **20** and not containing **1**, **5**, and **18**). The *B. rapa* samples of turnip greens and turnips showed also similar profiles (**2**, **4**, **6**−**10**, **12**, **13**, **15**, **18**, **19**, and **21**−**24**), but the glucosinalbin was not present in turnips, maybe due to their different origin of seeds. Garden cress and white mustard, which are closely related,[2] resulted also in similar GLS profiles. Radish presented **3**, **7**, **10**−**12**, **17**, **19**, **20**, and **22** GLS, quite different from the rest of the species.

Therefore, Brassicaceae sprouts showed characteristic GLSs according to species and their individual quantification (seeds; 4-, 8-, and 12-day-old sprouts; Tables 6−8). The general trend for the majority of the GLSs is decrease over germinated time, having a greater amount of the compound until day 4 (Tables 6−8), followed by a marked decline between days 4 and 12 (in broccoli, rutabaga, turnip greens, and radish), corresponding to 50−90% loss of individual GLSs. Kohlrabi, red cabbage, turnip, garden cress, and white mustard showed the highest loss of individual GLSs from seeds to day 8 of germination. Consistent with their function in plant defense and nutrient reserve compounds, seeds have the largest amount of these metabolites, and the reduction in GLSs with germination upon a dilution effect of tissue expansion leads to an intermediate GLS profile between seeds and mature tissues.[9,27] Not all of the GLSs found in seeds are detected in sprouts, as happened to sinigrin, glucoberteroin, or gluconasturtiin in broccoli, glucobrassicin in turnip greens, and progoitrin and glucoalyssin in garden cress (Tables 6−8), because the GLS profile may vary significantly between tissues and organs.[28] On the other hand, some GLSs were present only in sprouting seeds, such as neoglucobrassicin in broccoli, red cabbage, and white mustard sprouts (Tables 6 and 8). Several reasons might justify this fact, such as the activation of secondary metabolism during germination,[28] interconversion between aliphatic GLSs, or the interference between GLSs[27] and fatty nutrients in the seeds during sample extraction. Some authors have avoided this interference by defatting the samples.[8]

The values were recorded from 8-day-old-sprouts, being considered the optimum for consumption (suitable germination time to allow manipulation and acceptable composition by panelists and consumers). At this stage, broccoli and kohlrabi showed glucoraphanin (sulforaphane GLS), as the major GLS

Table 3. Data of Total Glucosinolates (mg 100 g^{-1} FW) Present in Brassicaceae Seeds and Sprouts[a]

	seeds	sprouts		
variety	D0	D4	D8	D12
broccoli	735.08e	209.32f	141.48e	117.45f
kohlrabi	1359.41c	994.40b	653.08b	450.54b
red cabbage	1307.78c	907.82c	516.42c	246.81c
rutabaga	2131.97b	951.88bc	386.84d	276.74c
turnip greens	1364.30c	736.66d	164.51e	119.44ef
turnip	1131.06d	938.63bc	766.07a	474.76b
radish	1350.76c	566.14e	296.77d	168.48de
garden cress	323.05f	194.94f	174.04e	176.32d
white mustard	2862.12a	2353.70a	815.10a	748.67a
$LSD_{0.05}$[b] (ANOVA $p < 0.001$)	37.40	26.96	24.47	14.24

[a]Mean values ($n = 3$). Different lower case letters indicate statistically significant differences among varieties (for each samplig day). [b]Least significant difference (LSD) for separating means in the respective column. The LSD was computed only after analysis of variance indicated a significant ($p < 0.05$) entry effect.

Table 4. Data of Total Phenolic Compounds (mg 100 g^{-1} FW) Present in Brassicaceae Seeds and Sprouts[a]

	seeds	sprouts		
variety	D0	D4	D8	D12
broccoli	1773.44d	1167.87d	832.16d	628.33e
kohlrabi	1149.34e	870.32e	823.58d	765.55bc
red cabbage	2116.64c	1321.31c	1309.29ab	991.92a
rutabaga	2200.86bc	1429.29c	828.50d	661.99de
turnip greens	2283.88b	1844.55b	743.59d	620.78e
turnip	1792.63d	1343.15c	1236.41b	706.23 cd
radish	3778.82a	2123.37a	1076.42c	751.89bc
garden cress	491.96f	516.65f	507.24e	422.49f
white mustard	182.27g	799.96e	779.25d	797.96b
$LSD_{0.05}$[b] (ANOVA $p < 0.001$)	39.67	41.07	36.16	19.04

[a]Mean values ($n = 3$). Different lower case letters indicate statistically significant differences among varieties (for each sampling day). [b]Least significant difference (LSD) for separating means in the respective column. The LSD was computed only after analysis of variance indicated a significant ($p < 0.05$) entry effect.

Table 5. Antioxidant Activity (mM Trolox 100 g^{-1} FW) of Brassicaceae Seeds and Sprouts Estimated by DPPH• Radical-Scavenging Assay and Ferric Reducing Antioxidant Assay (FRAP)[a]

	DPPH• assay					FRAP assay				
variety	seeds	D4	D8	D12	$LSD_{0.05}$[b]	seeds	D4	D8	D12	$LSD_{0.05}$[b]
broccoli	1.23bcA	0.47gB	0.28efC	0.21cdD	0.010	2.85cA	1.33fB	0.78cdC	0.63dD	0.053
kohlrabi	1.07deA	1.0aB	0.73cC	0.58aD	0.022	2.92bcA	2.46aB	1.61aC	1.18aD	0.044
red cabbage	1.51aA	0.95abB	0.77bC	0.40bD	0.035	3.43aA	2.08bB	1.61aC	1.00bD	0.066
rutabaga	1.12cdA	0.63eB	0.27fgC	0.21cdC	0.023	3.17bA	1.74cdB	0.81bcC	0.52eD	0.026
turnip greens	1.23bcA	0.70dB	0.24gC	0.18gD	0.017	2.75cdA	1.55deB	0.63deC	0.56deC	0.017
turnip	1.33bA	0.92bcB	0.71cC	0.46bD	0.022	2.51deA	1.84cB	1.57aC	0.96bD	0.057
radish	0.95eA	0.57fB	0.31eC	0.28cC	0.030	2.76cdA	1.42efB	0.98bC	0.76cD	0.068
garden cress	0.19gA	0.12hB	0.12hBC	0.09eD	0.010	0.52gA	0.49gA	0.45eB	0.32fC	0.012
white mustard	0.70 fA	0.64eB	0.36dC	0.24cdD	0.017	2.11fA	1.61deB	0.83bcC	0.76cC	0.060
$LSD_{0.05}$[b]	0.034	0.015	0.011	0.022		0.074	0.058	0.053	0.028	

[a]Mean values (n = 3). Different lower case letters indicate statistically significant differences between seeds and days (for each sampling day). Different upper case letters indicate statistically significant differences among varieties (for each variety). [b]Least significant difference (LSD) for separating means in the respective column. The LSD was computed only after analysis of variance indicated a significant ($p < 0.05$) entry effect. ANOVA values are significant at $p < 0.001$.

(35% of total) (Table 6). Broccoli also included other major GLSs, such as glucoerucin, 4-methoxyglucobrassicin, and neoglucobrassicin (15% of the total each), and kohlrabi showed glucoiberin (20% of the total) and glucoiberverin and 4-hydroxiglucobrassicin (10% of the total each). Red cabbage and rutabaga presented progoitrin, considered to be an antinutrient (goitrogenic effects), as the major GLS (35% of total). Red cabbage also included significant amounts of sinigrin (20% of the total) and glucoiberin and glucoiberverin (13% of the total each), and rutabaga presented gluconapin and 4-hydroxyglucobrassicin (25% of the total each) (Table 7). *B. rapa* varieties, turnip greens and turnips, exhibited gluconapin as characteristic GLS, with 75 and 50% of total, respectively (Table 7). Turnip greens presented <10% of the total for the rest of GLSs, and turnips also showed glucobrassicanapin (20% of the total) and 4-hydroxyglucobrassicin (14% of GLS). On the other hand, the also beneficial GLS glucoraphenin[14] was found to be dominant in radish (65% of total), showing also 4-hydroxyglucobrassicin as characteristic GLS (25% of the total) (Table 8). Finally, garden cress and white mustard presented a characteristic aromatic GLS, glucotropaeolin (80% of total) and glucosinalbin (87% of total), respectively, accounting for the rest of GLS, <10% of the total for both species. The total GLS content recorded in seeds (Table 3) was significantly higher and variable ($p < 0.001$) within species (from 2862.12 mg 100 g^{-1} FW in white mustard to 323.05 mg 100 g^{-1} FW in garden cress) than in sprouts. The GLSs recorded were higher during the first 4 days of germination, followed by a marked decrease over time, dropping from seeds to days 8 and 12 of germination, by 30 and 60%, respectively, in turnip; by 60 and 80%, respectively, in red cabbage; on average 60% in kohlrabi, garden cress, and white mustard sprouts; and around 80 and 85%, respectively, in broccoli, rutabaga, turnip greens, and radish sprouts (Table 3), also in agreement with previous results.[27] Our values are higher (2−15-fold) than those shown in studies with mature plants of broccoli, kohlrabi, or red cabbage[15] due to the physiological stage of sprouts. The differences also shown by other authors for broccoli, radish, and white mustard sprouts may be also due to the quality of the seeds used in the different works.[8,14,27] We also found similar results when comparing garden cress[29] and broccoli seeds of similar origin.[7]

Aliphatic GLSs (**1**−**7**, **9**−**11**, **13**, **15**−**18**, **20**, and **23** as shown in Tables 2 and 6−8, were the major GLSs in seeds and sprouts in all varieties (representing between 70 and 85%) (Figure 1), with values ranging from ∼1000 mg 100 g^{-1} FW in rutabaga seeds to ∼491 mg 100 g^{-1} FW in broccoli seeds, which decreased over the 12 day study period. Apart from glucoraphanin and glucoraphenin, the other predominant aliphatic GLS was sinigrin (which was mostly found in red cabbage, 91.83 ± 8.88 mg 100 g^{-1} FW, and kohlrabi, 28.99 ± 1.15 mg 100 g^{-1} FW), according to previous research (Tables 6−8).[14,19] Aliphatic GLSs are transformed by hydrolysis to isothiocyanates by specific myrosinases, which have been acknowledged as bioactive compounds with anticarcinogenic properties.[3,16] On the other hand, garden cress and white mustard exhibited a high content (90%) of aromatic GLSs (**8**, **14**, and **21** shown in Tables 2 and 6−8) in seeds (277 and ∼2749 mg 100 g^{-1} FW, respectively) and sprouts (Figure 1). Neither species showed any statistically significant difference among aromatic GLS concentration on 8- and 12-day-old sprouts (∼158 mg 100 g^{-1} FW in garden cress and ∼700 mg 100 g^{-1} FW in white mustard sprouts) (Table 8). The content of these GLSs provides a spicy taste, because the white mustard crop was bred for pungency as a condiment and now contains one of the highest concentrations reported of glucosinalbin in seeds (2749.53 mg 100 g^{-1} FW). For the rest of the cruciferous plants, aromatic GLSs accounted for <5% of the total GLSs. Indolic GLSs (**12**, **19**, **22**, and **24** shown in Table 2) in cruciferous seeds and sprouts showed values <30% of the total GLSs in all species except for garden cress and white mustard, which presented even much lower values (3.4 and 5%, respectively) (Figure 1). 4-Hydroxyglucobrassicin accounted for almost 90% of the indolic GLSs in all species. The process of germination resulted in a decreasing concentration of individual GLSs, except for the indole 4-methoxyglucobrassicin, which was found in trace amounts in all seeds, and some varieties presented high amounts of this GLS in growing sprouts (broccoli, red cabbage, rutabaga, turnip greens, radish, garden cress, and white mustard). In terms of biological effect, the expected breakdown product of the indole glucosinolate 4-methoxyglucobrassicin during ingestion, 4-methoxyindole-3-carbinol, has been studied because it might play a role in the cancer preventive effect by causing cell death and inhibiting cell proliferation of human colon cancer cells in vitro.[30]

Table 6. Individual Glucosinolates (mg 100 g^{-1} FW) Detected and Present in *Brassica oleracea* Seeds and Sprouts[a]

			broccoli					kohlrabi					red cabbage				
peak	code	compound	D0	D4	D8	12	LSD$_{0.05}$[b]	D0	D4	D8	D12	LSD$_{0.05}$	D0	D4	D8	D12	LSD$_{0.05}$
1	GIB	glucoiberin	241.03a	21.33b	20.02b	12.64b	8.72***	243.38a	187.45b	126.72c	103.37d	7.72***	161.02a	118.57b	73.46c	27.86d	5.17***
2	PRO	progoitrin	Tr	Tr	Tr	Tr		Tr	Tr	Tr	Tr		388.05a	458.44b	182.52c	99.43d	12.00***
3	GRE	glucoraphenin	nd	nd	nd	nd		nd	nd	nd	nd		nd	nd	nd	nd	
4	GRA	glucoraphanin	181.51a	80.05b	48.83c	42.39c	4.98***	522.13a	434.93b	264.57c	188.06d	9.43***	Tr	Tr	Tr	Tr	
5	EPRO	epiprogoitrin	nd	nd	nd	nd		nd	nd	nd	nd		Tr	Tr	Tr	Tr	
6	SIN	sinigrin	4.89	Tr	Tr	Tr		49.83a	46.12a	28.99b	21.87b	2.25***	224.43a	99.13b	91.83bc	44.54c	15.27***
7	GAL	glucoalyssin	Tr	Tr	Tr	Tr		Tr	Tr	Tr	Tr		Tr	Tr	Tr	Tr	
8	GSI	glucosinalbin	nd	Tr	Tr	Tr		Tr	nd	nd	nd		nd	nd	nd	nd	
9	GNL	gluconapoleiferin	nd	nd	nd	nd		nd	nd	nd	nd		nd	nd	nd	nd	
10	GNA	gluconapin	5.82a	1.17b	1.07b	0.97b	0.87**	55.79a	51.66a	25.09b	14.40c	2.44***	114.66a	70.43b	46.98c	17.89d	4.61***
11	GIV	glucoiberverin	Tr	Tr	Tr	Tr		223.10a	49.95b	65.48b	47.13b	6.83***	41.09b	67.03a	63.47a	28.33c	3.78***
12	OHGBS	4-hydroxyglucobrassicin	228.20a	9.53b	Tr	Tr	7.87***	181.68a	177.97a	120.39b	41.87c	8.10***	330.45a	144.98b	46.38c	17.07d	7.63***
13		*n*-butyl	nd	nd	nd	nd		nd	nd	nd	nd		nd	nd	nd	nd	
14	GTP	glucotropaeolin	nd	nd	nd	nd		nd	nd	nd	nd		nd	nd	nd	nd	
15	GBN	glucobrassicanapin	nd	nd	nd	nd		nd	nd	nd	nd		Tr	Tr	Tr	Tr	
16	GER	glucoerucin	46.61a	19.94b	18.39b	21.10b	3.34***	Tr	Tr	Tr	Tr		Tr	Tr	Tr	Tr	
17	DER	dehydoerucin	nd	nd	nd	nd		nd	nd	nd	nd		0	0	0	0	
18		*n*-pentyl	Tr	Tr	Tr	Tr		Tr	Tr	Tr	Tr		Tr	Tr	Tr	Tr	
19	GBS	glucobrassicin	14.63a	11.43ab	8.67b	7.96b	1.75**	28.13a	26.15a	26.45a	25.57a	1.06^{ns}	Tr	5.89a	2.78b	2.84b	0.25***
20	GBT	glucoberteroin	4.82	Tr	Tr	Tr		Tr	Tr	Tr	Tr		nd	nd	nd	nd	
21	GST	gluconasturtin	3.89	Tr	Tr	Tr		38.96a	12.92b	10.10bc	5.11c	2.39***	18.081a	11.82b	Tr	Tr	0.86***
22	MGBS	4-methoxyglucobrassicin	Tr	36.43a	18.64b	7.45c	2.08***	Tr	Tr	Tr	Tr		Tr	Tr	Tr	Tr	
23		*n*-hexyl	3.67a	4.57a	4.75a	4.74a	0.64^{ns}	16.41a	7.25b	5.29c	3.16d	0.31***	Tr	Tr	Tr	Tr	
24	NGBS	neoglucobrassicin	Tr	24.87a	21.15b	20.19b	0.72***	Tr	Tr	Tr	Tr		Tr	12.89a	9.00b	8.85b	0.89***

[a]Mean values (n = 3). Different lower case letters mean statistically significant differences between seeds and days (for each varieties). Tr, traces, not quantified (<0.5 mg 100 g^{-1} FW); nd, not detected (<0.1 mg 100 g^{-1} FW). [b]Least significant difference (LSD) for separating means in the respective column. The LSD was computed only after analysis of variance indicated a significant ($p < 0.05$) entry effect. ANOVA p value: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, $p > 0.05$.

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Table 7. Individual Glucosinolates (mg 100 g^{-1} FW) Detected and Present in *Brassica napus* (Rutabaga) and *Brassica rapa* (Turnip Greens and Turnip) Seeds and Sprouts[a]

			rutabaga					turnip greens					turnip				
peak	code	compound	D0	D4	D8	D12	$LSD_{0.05}$[b]	D0	D4	D8	D12	$LSD_{0.05}$	D0	D4	D8	D12	$LSD_{0.05}$
1	GIB	glucoiberin	nd	nd	nd	nd		nd	nd	nd	nd		nd	nd	nd	nd	
2	PRO	progoitrin	671.36a	281.04b	129.34c	98.49c	16.19***	45.00a	24.42b	13.08c	10.46c	2.99***	38.32a	28.57b	26.76b	13.07c	2.52***
3	GRE	glucoraphenin	nd	nd	nd	nd		nd	nd	nd	nd		nd	nd	nd	nd	
4	GRA	glucoraphanin	Tr	Tr	Tr	Tr		Tr	Tr	Tr	Tr		Tr	Tr	Tr	Tr	
5	EPRO	epiprogoitrin	nd	nd	nd	nd		nd	nd	nd	nd		nd	nd	nd	nd	
6	SIN	sinigrin	Tr	Tr	Tr	Tr		Tr	Tr	Tr	Tr		nd	nd	nd	nd	
7	GAL	glucoalyssin	132.46a	48.93b	8.76c	8.48c	7.77***	Tr	Tr	Tr	Tr		Tr	32.59a	23.80b	13.56c	2.43***
8	GSI	glucosinalbin	nd	nd	nd	nd		Tr	nd	nd	nd		nd	nd	nd	nd	
9	GNL	gluconapoleiferin	113.25a	93.86b	31.39c	31.48c	3.18***	Tr	nd	nd	nd		nd	nd	nd	nd	
10	GNA	gluconapin	684.75a	186.64b	89.57c	52.37d	10.05***	986.10a	542.39b	128.01c	99.64c	41.1***	565.92a	457.23b	396.79b	278.19c	19.88***
11	GIV	glucoiberverin	nd	nd	nd	nd		nd	nd	nd	nd		nd	nd	nd	nd	
12	OHGBS	4-hydroxyglucobrassicin	454.72a	284.35b	78.58c	38.25c	9.71***	249.89a	128.91b	18.25c	6.39c	8.57***	183.63a	156.27b	117.51c	61.59d	6.37***
13		*n*-butyl	Tr	Tr	Tr	Tr		Tr	Tr	Tr	Tr		nd	nd	nd	nd	
14	GTP	glucotropaeolin	nd	nd	nd	nd		nd	nd	nd	nd		nd	nd	nd	nd	
15	GBN	glucobrassicanapin	14.65a	7.16b	5.52c	2.95d	0.51***	13.94a	9.46b	5.17c	2.95d	0.86***	301.52a	223.87b	161.36c	95.81d	7.85***
16	GER	glucoerucin	nd	nd	nd	nd		nd	nd	nd	nd		nd	nd	nd	nd	
17	DER	dehydroerucin	nd	nd	nd	nd		nd	nd	nd	nd		nd	nd	nd	nd	
18		*n*-pentyl	nd	nd	nd	nd		Tr	Tr	Tr	Tr		Tr	nd	nd	nd	
19	GBS	glucobrassicin	10.99a	5.85b	2.64c	2.44c	0.38***	2.73	Tr	Tr	Tr		Tr	Tr	Tr	Tr	
20	GBT	glucoberteroin	Tr	Tr	Tr	Tr		nd	nd	nd	nd		nd	nd	nd	nd	
21	GST	gluconasturtin	49.79a	25.88b	23.76b	29.93b	4.81*	33.97a	10.39b	Tr	Tr	3.34***	41.68a	40.10a	39.84a	12.53b	4.72**
22	MGBS	4-methoxyglucobrassicin	nd	8.171c	17.28a	12.35b	0.91***	32.67a	20.72b	Tr	Tr	0.90***	Tr	Tr	Tr	Tr	
23		*n*-hexyl	Tr	Tr	Tr	Tr		Tr	Tr	Tr	Tr		Tr	Tr	Tr	Tr	
24	NGBS	neoglucobrassicin	Tr	Tr	Tr	Tr		Tr	Tr	Tr	Tr		Tr	Tr	Tr	Tr	

[a]Mean values ($n = 3$). Tr, traces, not quantified (<0.5 mg 100 g^{-1} FW); nd, not detected (<0.1 mg 100 g^{-1} FW). Different lower case letters indicate statistically significant differences between seeds and days (for each variety). [b]Least significant difference (LSD) for separating means in the respective column. The LSD was computed only after analysis of variance indicated a significant ($p < 0.05$) entry effect. ANOVA p value: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, $p > 0.05$.

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Table 8. Glucosinolates (mg 100 g^{-1} FW) Detected and Present in *Raphanus sativus* (Radish), *Lepidiym sativum* (Garden Cress), and *Sinapis alba* (White Mustard) Sprouts[a]

			radish					garden cress					white mustard				
peak	code	compound	D0	D4	D8	D12	$LSD_{0.05}$[c]	D0	D4	D8	D12	$LSD_{0.05}$	D0	D4	D8	D12	$LSD_{0.05}$
1	GIB	glucoiberin	nd	nd	nd	nd		Tr	nd	nd	nd		Tr	Tr	Tr	Tr	
2	PRO	progoitrin	nd	nd	nd	nd		27.45	Tr	Tr	Tr		nd	nd	nd	nd	
3	GRE	glucoraphenin	883.75[A]a	394.37b	197.54c	119.24d	8.97***	Tr	nd	nd	nd		nd	nd	nd	nd	
4	GRA	glucoraphanin	nd	nd	nd	nd		Tr	nd	nd	nd		Tr	Tr	Tr	Tr	
5	EPRO	epiprogoitrin	nd	nd	nd	nd		nd	nd	nd	nd		64.26a	63.97a	25.49b	20.55b	1.61***
6	SIN	sinigrin	nd	nd	nd	nd		Tr	Tr	Tr	Tr		Tr	Tr	Tr	Tr	
7	GAL	glucoalyssin	Tr	Tr	Tr	Tr		2.39	Tr	Tr	Tr		nd	nd	nd	nd	
8	GSI	glucosinalbin	nd	nd	nd	nd		11.54c	18.30a	15.80b	15.99b	0.61***	2749.53a	2200.42b	718.02c	650.66c	45.73***
9	GNL	gluconapoleiferin	nd	nd	nd	nd		Tr	nd	nd	nd		nd	nd	nd	nd	
10	GNA	gluconapin	Tr	Tr	5.84a	5.73a	0.68 [ns]	12.27a	10.23b	7.56c	7.47c	0.32***	nd	Tr	Tr	Tr	
11	GIV	glucoiberverin	Tr	Tr	Tr	Tr		nd	nd	nd	nd		nd	nd	nd	nd	
12	OHGBS	4-hydroxyglucobrassicin	426.16a	164.62b	74.03c	30.92d	5.71***	4.34	Tr	Tr	Tr		39.31a	27.58b	19.73c	19.31c	0.66***
13		*n*-butyl	nd	nd	nd	nd		nd	nd	nd	nd		nd	nd	nd	nd	
14	GTP	glucotropaeolin	nd	nd	nd	nd		265.06a	163.97b	141.61b	142.36b	8.81***	nd	6.16a	2.59b	2.36b	0.22***
15	GBN	glucobrassicanapin	nd	nd	nd	nd		nd	nd	nd	nd		nd	nd	nd	nd	
16	GER	glucoerucin	nd	nd	nd	nd		nd	nd	nd	nd		nd	nd	nd	nd	
17	DER	dehydoerucin	Tr	Tr	Tr	Tr		nd	nd	nd	nd		nd	nd	nd	nd	
18		*n*-pentyl	nd	nd	nd	nd		nd	nd	nd	nd		nd	nd	nd	nd	
19	GBS	glucobrassicin	Tr	Tr	Tr	Tr		Tr	Tr	Tr	Tr		9.01a	5.72b	3.16c	2.49c	0.52***
20	GBT	glucoberteroin	Tr	Tr	Tr	Tr		Tr	Tr	Tr	Tr		Tr	Tr	Tr	Tr	
21	GST	gluconasturtin	nd	nd	nd	nd		Tr	nd	nd	nd		nd	17.88a	17.14ab	16.54b	0.30***
22	MGBS	4-methoxyglucobrassicin	40.85a	7.15d	19.36b	12.59c	0.89***	Tr	2.44c	9.07b	10.50a	0.06***	nd	17.57b	21.46b	31.14a	1.13***
23		*n*-hexyl	nd	nd	nd	nd		nd	nd	nd	nd		nd	nd	nd	nd	
24	NGBS	neoglucobrassicin	nd	nd	nd	nd		Tr	Tr	Tr	Tr		nd	14.39a	7.51b	5.63b	0.84***

[a]Mean values ($n = 3$). Different lower case letters indicate statistically significant differences between seeds and days (for each variety). Tr, traces, not quantified (<0.5 mg 100 g^{-1} FW); nd, not detected (<0.1 mg 100 g^{-1} FW). [c]Least significant difference (LSD) for separating means in the respective column. The LSD was computed only after analysis of variance indicated a significant ($p < 0.05$) entry effect. ANOVA p value: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, $p > 0.05$.

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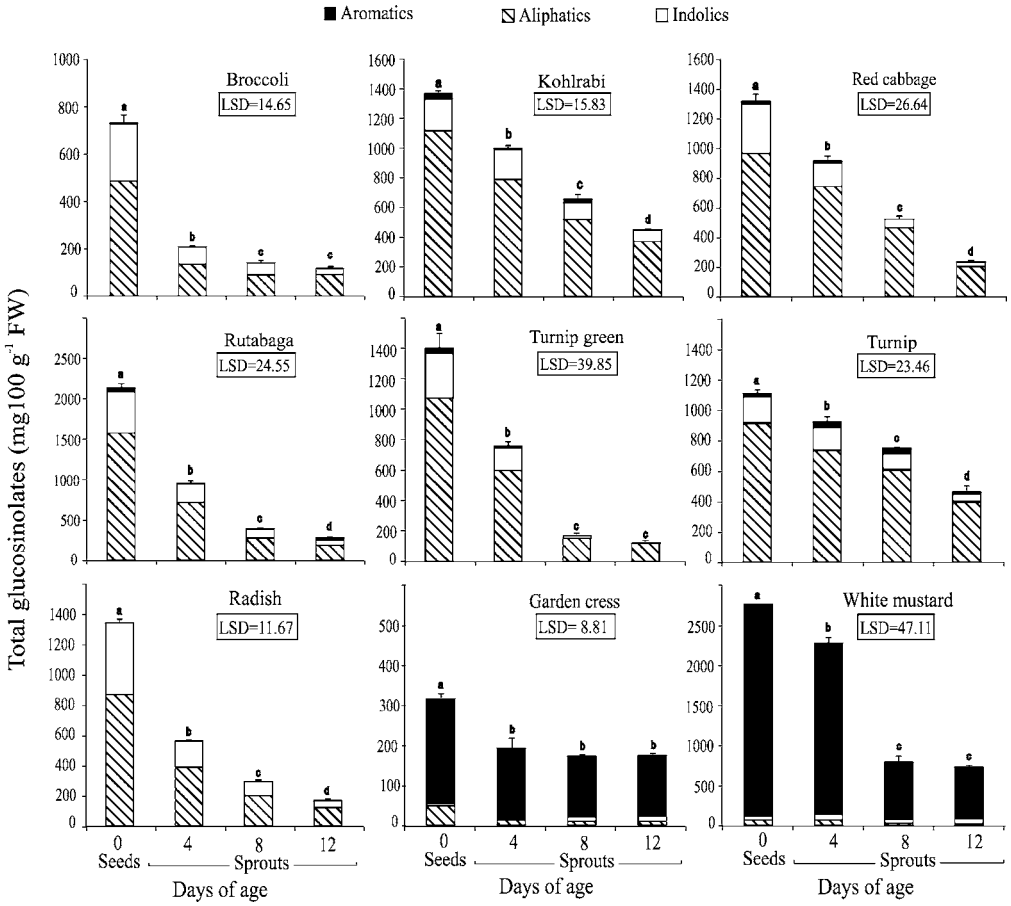

Figure 1. Aliphatic, indolic, and aromatic glucosinolates in cruciferous sprouts at 0, 4, 8, and 12 days after sowing. Values are the mean of three replicates representing mg 100 g^{-1} FW. Bars represent ±SD, and different symbols indicate significant differences between groups in the same parameter ($p < 0.05$).

In radish, the decreases of glucoraphenin and the increases of 4-methoxyblucobrassicin are convergent with data obtained by Ciska et al.[27] The age effect on the aliphatic, indolic, and aromatic GLSs showed little differences among varieties (Figure 1); because all species were cultivated under the same conditions, the observed variation in the level of total GLSs is expected to be mainly due to the genetic variation, as found by other authors,[15] as well as differences in characteristic individual GLSs in each species. Kohlrabi, red cabbage, turnip, and white mustard sprouts showed the highest amount of GLSs on days 8 and 12 of the germination period. The importance of GLSs, and more specially their hydrolysis products, in human health has been demonstrated by many researchers.[6,17,29] By selection of cruciferous crops, the level of desirable glucosinolates (i.e., glucoraphanin) can be enhanced considerably, which can lead to a substantial increase of the intake of health-promoting glucosinolates even without increasing the overall vegetable consumption. By contrast, a reduction of detrimental glucosinolates (progoitrin) has been carried out as a potential application for producing improved Brassicaceae vegetable breeding.[31] To reach this goal also the critical points in the finally consumed product (industrial processing and consumer preparation) have to be optimized and controlled.[15]

Phenolic Compounds. The phenolic composition of Brassicaceae vegetables has been recently investigated and, nowadays, the profile of the *Brassica* species is well established. The main classes of phenolic compounds found in crucifers were flavonols (mainly quercetin and kaempferol, but also isorhamnetin in some species) and hydroxycinnamic acids (specifically sinapic acid and chlorogenic acid derivatives). Phenolic compounds in seeds were significantly higher in content (Table 4) and variability ($p < 0.001$) than in sprouts (from ∼3778 mg 100 g^{-1} FW in radish and ∼1149 mg 100 g^{-1} FW in kohlrabi) except for garden cress and white mustard, which had lower values (∼ 492 and 182 mg 100 g^{-1} FW, respectively). These results in seeds showed differences among varieties and species suggesting the genotype as the main factor of variation. A decrease of phenolic compounds with growth was observed, although in terms of total contents, from seeds to days 8 and 12 of germination, by approximately 50 and 65%, respectively, in broccoli; by 30% in kohlrabi; by 35 and 55%, respectively, in red cabbage and turnip; and 70, 75, and 75% in rutabaga, turnip greens, and radish, respectively (Table 4). Garden cress showed similar values from seeds to 8-day-old sprouts (∼505 mg 100 g^{-1} FW), recording a decrease by 15% in 12-day-old sprouts. White mustard presented a 75% increase in total phenolics after sprouting. The sprouting seeds, due to their physiological stage,[35] showed higher values of total phenolics than commercial mature plants. The main phenolic compound group is the sinapic acid derivatives in seeds and sprouts.[33] These compounds accounted for >98% of the total phenolics, whereas flavonols and chlorogenic acid derivatives were <2% (Figure 2). In our study, sinapic acid derivatives accounted for approximately 70 and 80% in *B. rapa* seeds and sprouts, respectively, but showed higher values of flavonols in seeds (25−30% of total phenolics) and sprouts (21% in 8-day-old

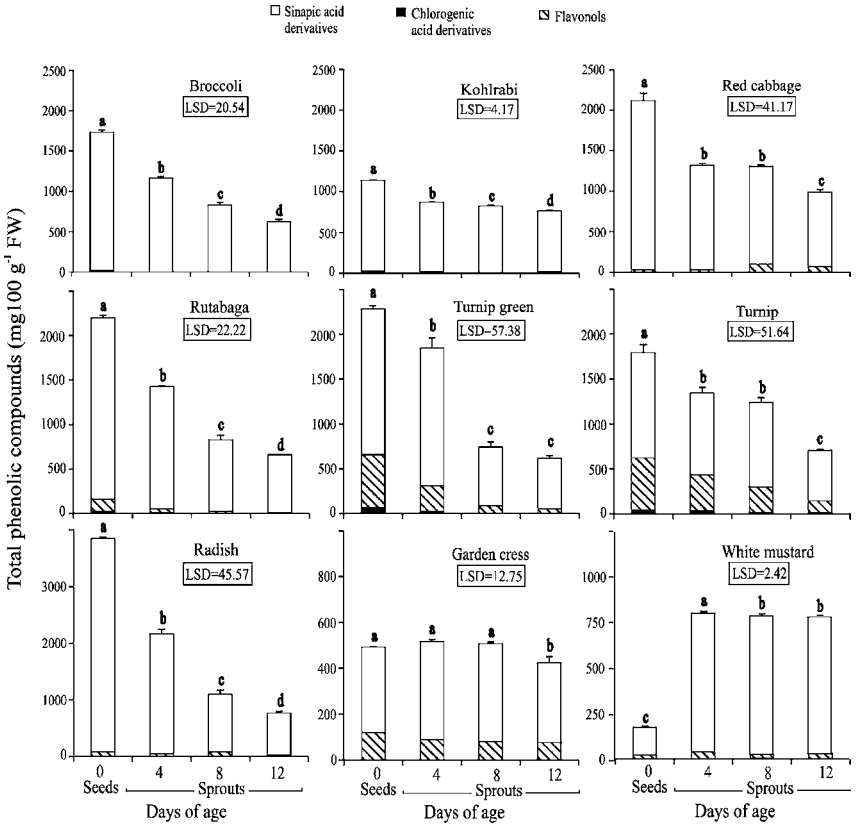

Figure 2. Sinapic acid derivatives, flavonols, and chlorogenic acid derivatives in cruciferous sprouts at 0, 4, 8, and 12 days after sowing. Values are the mean of three replicates representing mg 100 g^{-1} FW. Bars represent ±SD, and different symbols indicate significant differences between groups in the same parameter ($p < 0.05$).

sprouts of turnip; 11% in 8-day-old sprouts of turnip greens). These values may be associated with the presence of isorhamnetin, a flavonol that is almost absent in *B. oleraceae*.[32] Chlorogenic acid derivatives were reported between 1 and 4% in turnip crops, recording higher values in seeds. Rutabaga, radish, and white mustard also registered high values of sinapic acid derivatives (~90%), as well as flavonols (from 2 to 6% in seeds and from 2 to 7% in 8-day-old sprouts) and chlorogenic acid derivatives (~1%). Garden cress seeds and sprouts recorded between 75 and 80% of sinapic acid derivatives and between 15 and 20% of flavonols, containing traces of chlorogenic acid derivatives (Figure 2). According to earlier works,[33] red cabbage seems to be a very good source of phenolics among *B. oleraceae*, with values similar to those found in turnips on day 8 of germination (~1300 mg 100 g^{-1} FW total phenolics). After 12 days of sprouting, also radish (752 mg 100 g^{-1} FW) and kohlrabi (766 mg 100 g^{-1} FW) showed high values of total phenolics. In our results, and according to other authors,[20,26,33−35] hydroxycinnamic acids are predominant phenolics, and flavonols were found in lower concentrations. The distribution of phenolic compounds was variable according to the variety evaluated.

In Vitro Antioxidant Capacity. Comparison of antioxidant capacity between varieties was used as a comparison criterion in the study, and it is also useful to correlate with the phenolic compounds in the sprouts and seeds. Similarly to what was previously found, the antioxidant activity of the vegetables largely depends on growth conditions.[35,36] Two different methods to evaluate the antioxidant capacity were used: a free radical scavenging method (DPPH• assay) and a ferric reducing antioxidant potential (FRAP) assay (Table 5). These methods have been widely used because they require relatively standard equipment and provide rapid and reproducible results. Antioxidant activity values obtained with the FRAP assay were higher than those obtained with the DPPH• assay (Table 5), coinciding with Ali et al.[37] All species tested showed a decrease of the antioxidant capacity during the germination period. The activity expressed on a fresh weight basis (FW) may be influenced by the dilution effect. Statistically significant variations among species for seeds and sprouts for the DPPH• assay were found, with values ranging from 1.51 to 0.19 mM Trolox g^{-1} FW in seeds, and for the FRAP assay, these values ranged from 2.08 to 0.49 mM Trolox g^{-1} FW (Table 5). These values are similar to those previously reported for broccoli sprouts,[7] cabbage,[36] and radish.[38] As for germinating seeds, 4-day-old sprouts provided the highest values of antioxidant capacity (from 1.00 to 0.12 mM Trolox g^{-1} FW in the DPPH• assay and from 2.46 to 0.49 mM Trolox g^{-1} FW in the FRAP assay). Because a minimum of 8 days of growth is necessary to provide commercial edible sprouts, at this point red cabbage, turnip, and kohlrabi were the varieties with the highest values of antioxidant capacity, around 0.75 and 1.60 mM Trolox g^{-1} FW on day 8 and 0.50 and 1.00 mM Trolox g^{-1} FW on day 12, obtained by the DPPH• and FRAP assays, respectively. Results exhibited relatively significant ($p < 0.01$) correlation between values of total phenolics and antioxidant capacity ($r^2 = 0.686$ for the DPPH• assay and $r^2 = 0.712$ for the FRAP assay). Because sinapic acid derivatives were the predominant group of phenolic compounds analyzed, similar values for correlation with antioxidant capacity were found. The trend for both assays of the nine sprout varieties tested did not vary markedly, and a significant correlation

($p < 0.001$) between methods ($r^2 = 0.965$) was found, in agreement with Dudonné et al.,[39] who reported $r^2 = 0.822$. These values of antioxidant capacity of sprouts reached a 10-fold increase compared to commercial adult plants studied by different authors.[36,39] Some previously published results indicated similar values in broccoli sprouts[7] and radish sprouts.[38] In agreement with Podsedek et al.,[34] red cabbage belongs to the group of *Brassica* species with higher antioxidant capacity. Phenolic compounds are the major natural antioxidants of crucifers, and in broccoli,[40] it was reported they were responsible for 80−95% of the total antioxidant capacity.

To summarize, Brassicaceae sprouts are foods rich in glucosinolates and natural antioxidants. The differences observed in GLS profiling among genotypes are both qualitative and quantitative, finding characteristic GLSs in different species. The phenolic compounds also showed significant differences between varieties in accordance with previous results.[32,35] The sprouts with better biomass ratio should be selected (i.e., red cabbage and radish) also with higher glucosinolates, phenolics, and antioxidant capacity. On the other hand, white mustard, turnips, or kohlrabi, having the highest concentrations of glucosinolates, showed lower values of biomass.

The selection of suitable varieties and the germination time, 8- and 12-day-old sprouts, for biomass and size is important to maximize the health-promoting properties of the sprouts, even without increasing the overall vegetable consumption. To reach this goal, also critical points of industrial processing and consumer preparation need to be optimized.[15] Further research is guaranteed for the understanding of the bioavailability and metabolism of these phytochemicals to allow scientifically backed statements and recommendations for dietary intake, effective dosages, and dietary guidelines for nutrition and health.

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Notes

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