Integration of good practices and new methods for professional

Training in the field of herbs processing for food and food supplements 2014-1-RO01-KA200-002902

CHAPTER 16.

STUDIES OF NOVEL CRUCIFEROUS SPROUTS RICH IN BIOACTIVE COMPOUNDS

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CONTENT

1.INTRODUCTION

Brassicaceae vegetables include a variety of horticultural crops with global economical relevance (oilseeds, forage, condiments, and vegetables). Some species very known are *B. oleracea* (such as kale, cabbage, broccoli and kohlrabi), *B. rapa* (such as turnip and Chinese cabbage), *B. 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, improving immune system, against allergies, showing antihypertensive properties and reducing the risk for cardiovascular diseases, and certain types of cancer (Kim et al., 2009; Van Horn, et al., 2008). 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. The content of bioactive compounds in *Brassica*ceae vegetables varies with genotype, environmental stress and growth conditions, storage, processing and cooking methods. 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. Glucosinolates are classified as aliphatic (the major group in almost all crucifers seeds and sprouts of *B. oleraceae, B napus, B. rapa and R. sativus),* indolic (representing lower amount in the glucosinolate profile), or aromatic (characteristic in *Sinapis alba* and *Lepidium sativum*), and have been extensively studied due to their hydrolysis compounds, the isothiocyanates (such as sulphoraphane and benzyl isothiocyanate) and indoles (indol-3-carbinol) which are associated with a reduced risk for particularly cancers of gastrointestinal tract, lung and prostate (Fahey, et al., 1999). The phenolic profile of sprouts is composed mostly of sinapic acid derivatives (hydroxycinnamic acids), a small portion of flavonoids (mainly quercetin and kaempferol and antocyanins) and other hydroxycinnamic acids (chlorogenic, *p*-coumaric, and ferulic acids and their derivatives) (Francisco et al., 2009). *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. The aim of the present work was to characterize 9 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.

2. PLANT MATERIAL AND EXPERIMENTAL CONDITIONS

Seeds were rinsed in distilled water, immersed in 5 gL^{-1} sodium hypochlorite in distilled water under aeration overnight. After pouring off the soaking water, the seeds were weighed (day 0) and spreaded evenly on trays (5 g per tray) lined with cellulose. 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 ºC, respectively. *Brassicaceae* sprouts were allowed to grow until they reached 12 days of age. Sprout samples were collected at different time points after germination (days 4, 8, and 12). All samples were weighed (fresh mass), collected separately, flash frozen in liquid nitrogen, and stored at −80 ºC prior to analyses.

3. EXTRACTION AND DETERMINATION OF GLUCOSINOLATES AND PHENOLIC COMPOUNDS

Freeze-dried samples (100 mg) were extracted with 1.5mL of 700 gL^{-1} methanol, heated at 70ºC for 30 min and centrifuged (17 500 × *g*, 30min, 4ºC). The supernatants were collected and methanol was completely removed using a rotary evaporator. The dry material obtained was dissolved in 1mL of ultrapure water and filtered through a 0.45 µm Millex-HV13 membrane. Caffeoyl-quinic acid derivatives were quantified as chlorogenic acid (5-caffeoyl-quinic acid, Sigma, St Louis, MO, USA), flavonoids as quercetin 3- rutinoside (Sigma, St Louis, MO, USA) and sinapic acid and ferulic derivatives as sinapic acid (Sigma, St Louis, MO, USA). The total analyte content of phenolic compounds in broccoli sprouts was expressed as mg per 100g FW.

4. 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 Francisco et al., 2009. Glucosinolates were quantified in the HPLC-PDA using sinigrin as standard (sinigrin monohydrate, Phytoplan Diehm & Neuberger, GmbH, Heidelberg, Germany). Caffeoyl-quinic acid derivatives were quantified as chlorogenic acid (5-caffeolyl-quinic 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, St Louis, MO, USA).

5. STATISTICAL METHODS

All assays were conducted by triplicate. The data were processed using the SPSS 17.0 software package (LEAD Technologies, Inc., Chicago, USA). A Student's t-test was used to determine the significance of differences between means. A multifactorial analysis of variance (ANOVA) and the Tukey's Multiple Range Test were carried out to determine significant differences at P values < 0.05.

6. BIOMASS

Table 1 shows an increasing biomass ratio from day 0 to 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). The day 8 of the monitored period, broccoli, rutabaga, turnip greens and radish, had a biomass ratio significantly higher than the rest (2-3 folds), consistent with the greater length (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 production.

Table 1. Data of biomass increase ratio (sprouts *vs*. seeds) in *Brassicaceae* sprouts.

Varieties	Scientific name	% Germination	Day 4	Day 8	Day 12	ANOVA [C]	$LSD_{0.05}$ [B]
Broccoli	*Brassica oleracea* var. *italica*	> 90	2.18[A]a	3.17a	3.33a	n.s.	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	0.29cd	0.57c	0.87bc	n.s.	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
$LSD_{0.05}$ [B](ANOVA P<0.001)			0.15	0.37	0.25		

[A]Mean values (n=3).

[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.

[C]Levels of significance for each sampling day between species. Non-significant at P>0.05 (n.s.); significant at P<0.05 (*); significant at P<0.01 (**); significant at p<0.001 (***).

a-h, Different lowercase-letters mean statistically significant differences among varieties (for each sampling day).

A-C Different uppercase-letters mean statistically significant differences between days (for each varieties).

7.GLUCOSINOLATES

Table 2. List of glucosinolates detected and presented in *Brassicaceae* seeds and sprouts.

Peak	Code	Compound	Glucosinolate (gls) semisystematic name	Classe	Rt [min]	[M-H]$^-$ (*m/z*)	Broccoli	Kohlrabi	Red cabbage	Rutabaga	Turnip green	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	Aliphat	12.7	450	+	+	+	+	+	+	+	+	0

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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-Hydroxiglucobrassicin	4-hydrony-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-methyltio-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	+	+

Identification based in $[M-H]^-$ (*m/z*), retention time (Rt) and characteristic spectra. +, compounds presence. 0, compound absence.

Results showed significant differences of the characteristic GLSs profiles among samples (Table 2).

Brassicaceae sprouts showed characteristic GLSs according to species and their individual quantification (seeds, 4, 8 and 12-days-old sprouts; Tables 3, 4 and 5).

Table 3. List of individual glucosinolates (mg 100g^{-1} F.W.) detected and presented in *Brassica oleracea* seeds and sprouts.

Peak	Code	Compound	Broccoli D0	Broccoli D4	Broccoli D8	Broccoli D12	LSD$_{0.05}$^{B}	Kohlrabi D0	Kohlrabi D4	Kohlrabi D8	Kohlrabi D12	LSD $_{0.05}$	Red cabbage D0	Red cabbage D4	Red cabbage D8	Red cabbage D12	LSD $_{0.05}$
1	GIB	Glucoiberin	241.03^{A}a	21.33 b	20.02b	12.64b	8.72^{***}	243.38a	187.45 b	126.72c	103.37d	7.72^{***}	161.02 a	118.57 b	73.46 c	27.86d	5.17^{***}
2	PRO	Progoitrin	Tr	Tr	Tr	Tr		Tr	Tr	Tr	Tr		388.05 a	458.44 b	182.52c	99.43d	12.00^{***}
3	GRE	Glucoraphenin	n.d.	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.	
4	GRA	Glucoraphanin	181.51 a	80.05 b	48.83c	42.39c	4.98^{***}	522.13a	434.93 b	264.57c	188.06d	9.43^{***}	Tr	Tr	Tr	Tr	
5	EPRO	Epiprogoitrin	n.d.	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.		Tr	Tr	Tr	Tr	
6	SIN	Sinigrin	4.89	Tr	Tr	Tr		49.83a	46.12a	28.99	21.87		224.43	99.13b	91.83	44.54c	15.2

										b	b	2.25***	a		bc		7***
7	GAL	Glucoalyssin	Tr	Tr	Tr	Tr		Tr	Tr	Tr	Tr		Tr	Tr	Tr	Tr	
8	GSI	Glucosinalbin	n.d.	Tr	Tr	Tr		Tr	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.	
9	GNL	Gluconapoleiferin	n.d.	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.	
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-Hydroxiglucobrassicin	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	n.d.	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.	
14	GTP	Glucotropaeolin	n.d.	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.	
15	GBN	Glucobrassicanapin	n.d.	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.		Tr	Tr	Tr	Tr	
16	GER	Glucoerucin	46.61a	19.94	18.39b	21.10b		Tr	Tr	Tr	Tr		Tr	Tr	Tr	Tr	

				b			3.34^{***}										
17	DER	Dehydoerucin	n.d.	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.		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	$1.06^{n.s}$	Tr	5.89a	2.78b	2.84b	0.25^{***}
20	GBT	Glucoberteroin	4.82	Tr	Tr	Tr		Tr	Tr	Tr	Tr		n.d.	n.d.	n.d.	n.d.	
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	$0.64^{n.s}$	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) Tr, traces, no quantified (<0.5 mg $100g^{-1}$ F.W.). n.d., not detected (<0.1 mg $100g^{-1}$ F.W). a-d, Different lowercase-letters mean statistically significant differences between seeds and days (for each varieties). [B], Least Dignificant 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, * $p<0.05$; ** $p<0.01$; *** $p<0.001$; n.s. $p>0.05$

Table 4. List of individual glucosinolates (mg 100g^{-1} F.W.) detected and presented in *Brassica napus* (rutabaga*) and Brassica rapa* (turnip green and turnip) seeds an

Peak	Code	Compound	Rutabaga D0	Rutabaga D4	Rutabaga D8	Rutabaga D12	$LSD_{0.05}$^{B}	Turnip green D0	Turnip green D4	Turnip green D8	Turnip green D12	$LSD_{0.05}$	Turnip D0	Turnip D4	Turnip D8	Turnip D12	$LSD_{0.05}$
1	GIB	Glucoiberin	n.d.	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.	
2	PRO	Progoitrin	671.36^{A}a	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	n.d.	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.	
4	GRA	Glucoraphanin	Tr	Tr	Tr	Tr		Tr	Tr	Tr	Tr		Tr	Tr	Tr	Tr	
5	EPRO	Epiprogoitrin	n.d.	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.	
6	SIN	Sinigrin	Tr	Tr	Tr	Tr		Tr	Tr	Tr	Tr		n.d.	n.d.	n.d.	n.d.	
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	n.d.	n.d.	n.d.	n.d.		Tr	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.	
9	GNL	Gluconapoleiferin	113.25a	93.86b	31.39c	31.48c	3.18***	Tr	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.	
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***

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11	GIV	Glucoiberverin	n.d.	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.	
12	OHGBS	4-Hydroxiglucobrassicin	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		n.d.	n.d.	n.d.	n.d.	
14	GTP	Glucotropaeolin	n.d.	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.	
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	n.d.	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.	
17	DER	Dehydoerucin	n.d.	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.	
18		n-pentyl	n.d.	n.d.	n.d.	n.d.		Tr	Tr	Tr	Tr		Tr	n.d.	n.d.	n.d.	
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		n.d.	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.	
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	MGB	4-	n.d.	8.171	17.28a	12.35b	0.91**	32.67a	20.72b	Tr	Tr		Tr	Tr	Tr	Tr	

	S	Methoxyglucobrassicin		c			*					0.90 ***				
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, no quantified (<0.5 mg 100g^{-1} F.W.). n.d., not detected (<0.1 mg 100g^{-1} F.W).

a-d, Different lowercase-letters mean statistically significant differences between seeds and days (for each varieties).

[B], Least Dignificant 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, * $p<0.05$; ** $p<0.01$; *** $p<0.001$; n.s. $p>0.05$

Table 5. List of glucosinolates (mg 100g^{-1} F.W.) detected and presented in *Raphanus sativus* (Radish), *Lepidiym sativum* (garden cress) *and Sinapis alba* (white mu

Peak	Code	Compound	Radish D0	Radish D4	Radish D8	Radish D12	LSD$_{0.05}$ [B]	Garden cress D0	Garden cress D4	Garden cress D8	Garden cress D12	LSD $_{0.05}$	White mustard D0	White mustard D4	White mustard D8	White mustard D12	LSD $_{0.05}$
1	GIB	Glucoiberin	n.d.	n.d.	n.d.	n.d.		Tr	n.d.	n.d.	n.d.		Tr	Tr	Tr	Tr	
2	PRO	Progoitrin	n.d.	n.d.	n.d.	n.d.		27.45	Tr	Tr	Tr		n.d.	n.d.	n.d.	n.d.	
3	GRE	Glucoraphenin	883.75 [A]a	394.37b	197.54c	119.24d	8.97***	Tr	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.	
4	GRA	Glucoraphanin	n.d.	n.d.	n.d.	n.d.		Tr	n.d.	n.d.	n.d.		Tr	Tr	Tr	Tr	
5	EPRO	Epiprogoitrin	n.d.	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.		64.26a	63.97a	25.49b	20.55b	1.61***
6	SIN	Sinigrin	n.d.	n.d.	n.d.	n.d.		Tr	Tr	Tr	Tr		Tr	Tr	Tr	Tr	
7	GAL	Glucoalyssin	Tr	Tr	Tr	Tr		2.39	Tr	Tr	Tr		n.d.	n.d.	n.d.	n.d.	
8	GSI	Glucosinalbin	n.d.	n.d.	n.d.	n.d.		11.54c	18.30a	15.80b	15.99b	0.61***	2749.53a	2200.42b	718.02c	650.66c	45.73***
9	GNL	Gluconapoleiferin	n.d.	n.d.	n.d.	n.d.		Tr	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.	

10	GNA	Gluconapin	Tr	Tr	5.84a	5.73a	0.68$^{n.s}$	12.27a	10.23b	7.56c	7.47c	0.32***	n.d.	Tr	Tr	Tr	
11	GIV	Glucoiberverin	Tr	Tr	Tr	Tr		n.d.	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.	
12	OHGBS	4-Hydroxiglucobrassicin	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	n.d.	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.	
14	GTP	Glucotropaeolin	n.d.	n.d.	n.d.	n.d.		265.06a	163.97b	141.61b	142.36b	8.81***	n.d.	6.16a	2.59b	2.36b	0.22***
15	GBN	Glucobrassicanapin	n.d.	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.	
16	GER	Glucoerucin	n.d.	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.	
17	DER	Dehydoerucin	Tr	Tr	Tr	Tr		n.d.	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.	
18		n-pentyl	n.d.	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.	
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	n.d.	n.d.	n.d.	n.d.		Tr	n.d.	n.d.	n.d.		n.d.	17.88a	17.14	16.54b	0.30*

															ab		**
22	MGBS	4-Methoxyglucobrassicin	40.85a	7.15d	19.36b	12.59c	0.89***	Tr	2.44c	9.07b	10.50a	0.06***	n.d.	17.57b	21.46b	31.14a	1.13***
23		n-hexyl	n.d.	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.	
24	NGBS	Neoglucobrassicin	n.d.	n.d.	n.d.	n.d.		Tr	Tr	Tr	Tr		n.d.	14.39a	7.51b	5.63b	0.84***

[A]Mean values (n=3) Tr, traces, no quantified (<0.5 mg 100g^{-1} F.W.). n.d., not detected (<0.1 mg 100g^{-1} F.W).

a-d, Different lowercase-letters mean statistically significant differences between seeds and days (for each varieties).

[B], Least Dignificant 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; n.s. p>0.05

The general trend for the majority of the GLSs is decrease over germinated time, having greater amount of the compound until day 4 (Tables 3, 4, 5), followed by a marked decline between day 4 and 12 (in broccoli, rutabaga, turnip green and radish), corresponding to 50-90% loss of individual GLSs. 8-days-old-sprouts were 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 major GLS (35% of total) (Table 3, 5). Red cabbage and rutabaga presented progoitrin, considered antinutrient (goitrogenic effects), as major GLS (35% of total) (Table 3-5). *Brassica rapa* varieties, turnip greens and turnips, exhibited gluconapin as characteristic GLS, with 75% and 50% of total, respectively (Table 4, 5). On the other hand, the also beneficial GLS glucoraphanin (Martínez-Villaluenga, et al., 2010), was found to be the dominant in radish (65% of total), showing also 4-hydroxiglucobrassicin as characteristic GLS (25% of the total) (Table 5). Finally, garden cress and white mustard presented a characteristic aromatic GLS, glucotropaeolin (80% of total), and glucosinalbin (87% of total), respectively, accounting the rest of GLS <10% of the total for both species. The total GLSs content recorded in seeds (table 5) was significant higher and variable ($P<0.001$) within species (from 2862.12 in white mustard to 323.05 mg·100 g^{-1} F.W. in garden cress) than in sprouts.

Table 5. Data of total glucosinolates (mg 100g^{-1} F.W.) presents in *Brassicaceae* seeds and sprouts.

Varieties	Seeds	Sprouts		
	Day 0	Day 4	Day 8	Day 12
Broccoli	735.08[A] e	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 green	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

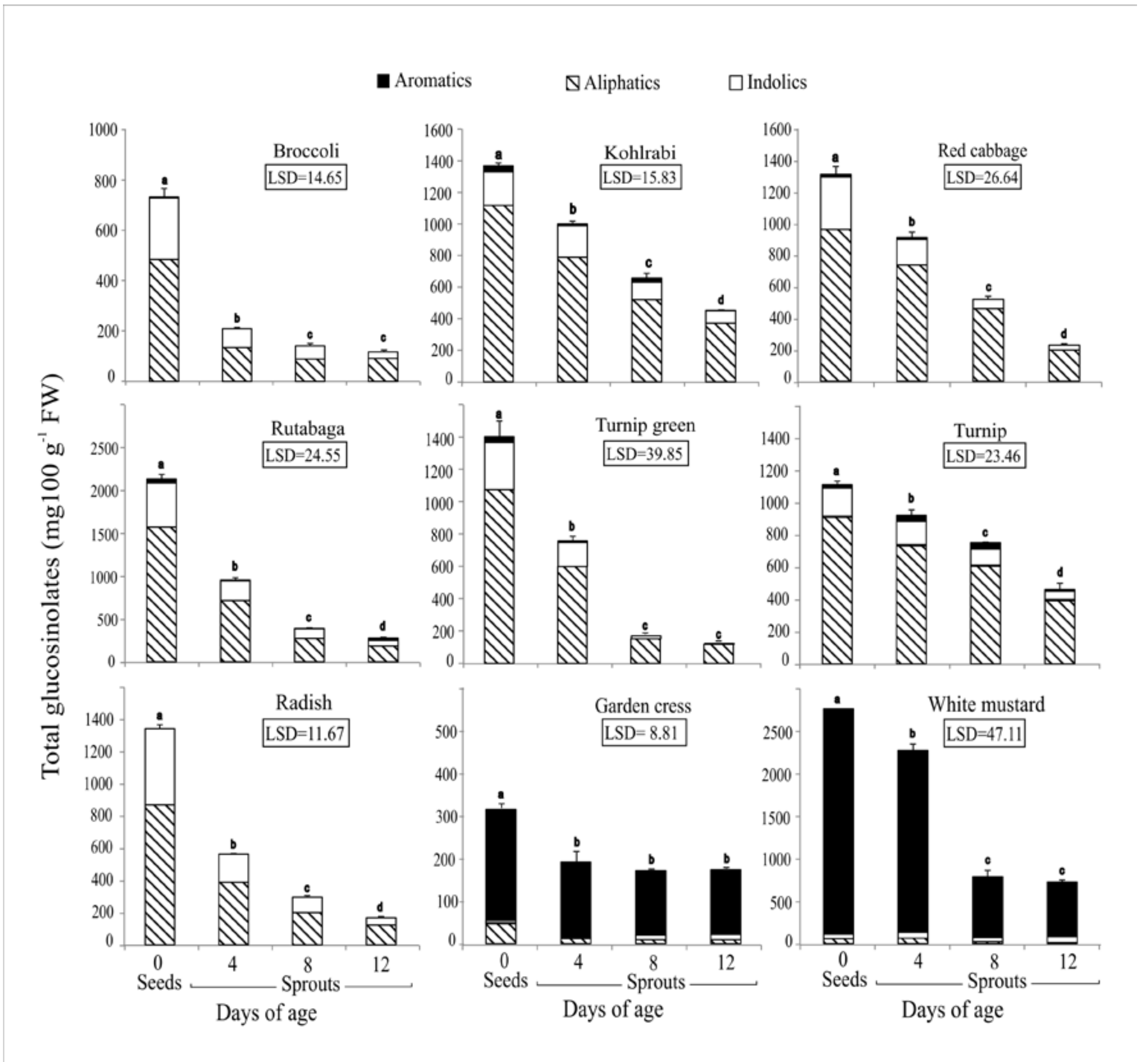
[A]Mean values (n=3)

[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.

a-h, Different lowercase-letters mean statistically significant differences among varieties (for each samplig day).

Aliphatic GLSs as showed in Table 2, 3, 4 and 5, were the major GLSs in seeds and sprouts in almost all varieties (representing between 70% - 85%) (Figure 1). Aliphatic GLS are transformed by hydrolysis to isothiocyanates by specific myrosinases, which have been acknowledged as bioactive compounds with anticarcinogenic properties (Kim, et al, 2009; Fahey, et al, 1999). On the other hand, garden cress and white mustard exhibited high content (90 %) of aromatic GLSs (Figure 1). Indolic GLSs 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).

Figure 1. Aliphatic, indolic and aromatic glucosinolates in cruciferous sprouts at 0, 4, 8 and 12 after sowing. Values are mean of three replicates represented mg $\cdot$100 g^{-1} F.W. Bars represent +SD and different symbols indicate significant differences between groups in the same parameter (*P < 0.05).*



Aromatics
Aliphatics
Indolics
Total glucosinolates (mg100 g-1 FW)
Broccoli
LSD=14.65
Kohlrabi
LSD=15.83
Red cabbage
LSD=26.64
Rutabaga
LSD=24.55
Turnip green
LSD=39.85
Turnip
LSD=23.46
Radish
LSD=11.67
Garden cress
LSD= 8.81
White mustard
LSD=47.11
Seeds
Sprouts
Days of age

8.PHENOLIC COMPOUNDS

The main classes of phenolic compounds found in crucifers were flavonols (mainly quercetin and kaempferol) and hydroxycinnamic acids (specifically sinapic acid and chlorogenic acid derivatives). Phenolic compounds in seeds were significantly higher in content (Table 7) than in sprouts, except for garden cress and white mustard, which had lower values. A decrease of phenolic compounds with growth was observed, from seeds to day 8 and day 12 of germination, in all species. The sprouting seeds, due to their physiological stage (Singh, et al., 2007), showed higher values of total phenolics than commercial mature plants. The main phenolic compounds group is the sinapic acid derivatives in seeds and sprouts (Cartea, et al., 2011). These compounds accounted for more than 98% of the total phenolics, whereas flavonols and chlorogenic acid derivatives were less than 2% (Figure 2).

Table 7. Data of total phenolic compounds (mg $100g^{-1}$ F.W.) presents in *Brassicaceae* seeds and sprouts.

	Seeds	Sprouts		
Varieties	Day 0	Day 4	Day 8	Day 12
Broccoli	1773.44 [A] d	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 green	2283.88b	1844.55b	743.59d	620.78e
Turnip	1792.63d	1343.15c	1236.41b	706.23cd
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

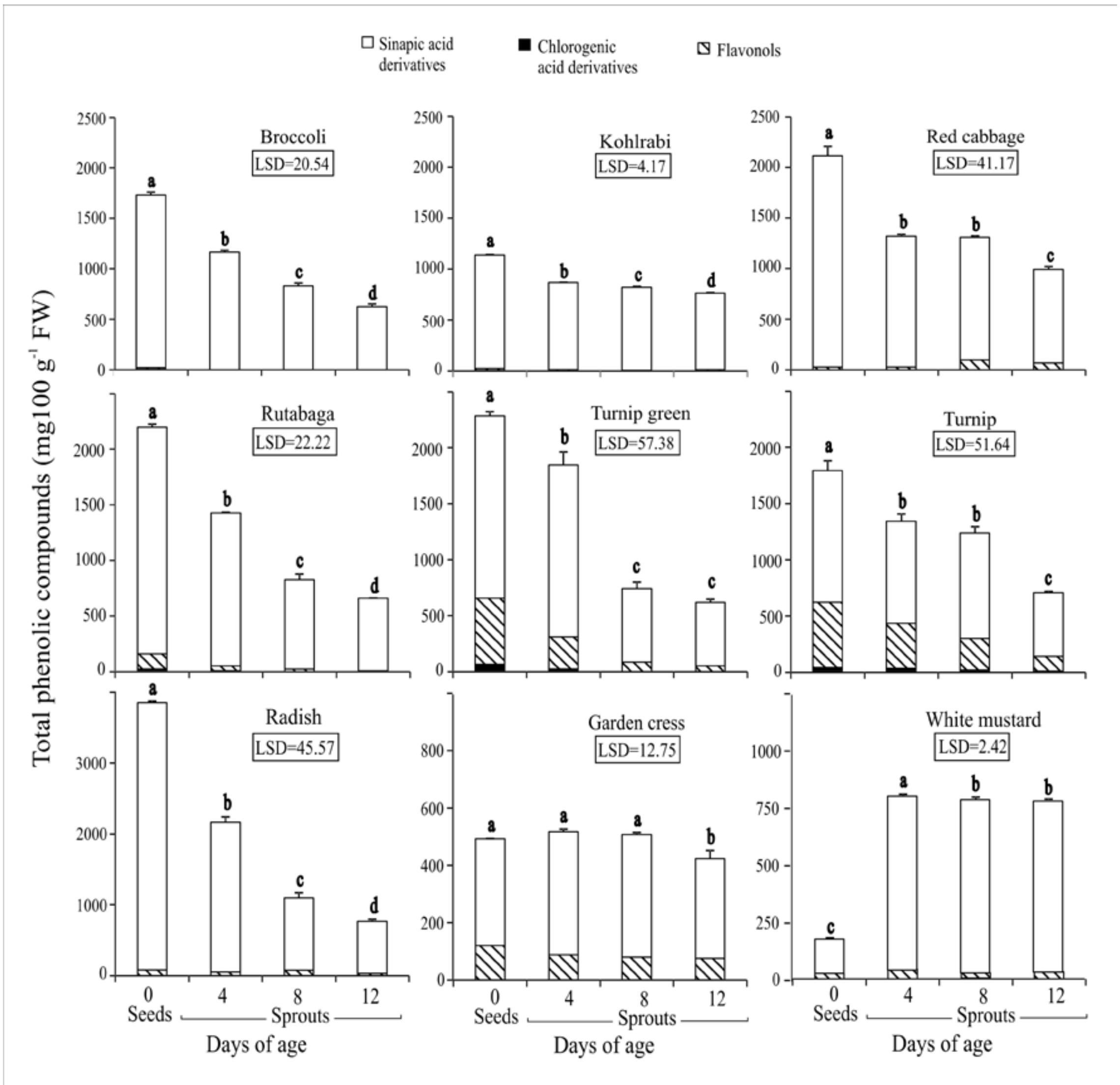
[A]Mean values (n=3)

[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.

a-h, Different lowercase-letters mean statistically significant differences among varieties (for

each samplig day).

Figure 2. Sinapic acid derivatives, flavonols and chlorogenic acid derivatives in cruciferous sprouts at 0, 4, 8 and 12 after sowing. Values are mean of three replicates represented mg ·100 g^{-1} F.W. Bars represent +SD and different symbols indicate significant differences between groups in the same parameter (*P < 0.05).*



9. CONCLUSIONS

To summarize, *Brassicaceae* sprouts are foods rich in glucosinolates and natural antioxidants. The differences observed in GLSs profiling among genotypes are both qualitative and quantitative, finding characteristic GLSs in different species. The sprouts with better biomass ratio should be selected (broccoli and radish) also with higher glucosinolates, phenolics and antioxidant capacity. 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.

10. ACKNOWLEDGEMENTS

The authors are grateful to the CICYT (Project AGL2009 – 12720) and the Region of Murcia – Science and Technology Agency – Fundación Séneca (´Excelence in Research Group' Exp. 04486/GERM/06 and Project 11909/PI/09).

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