JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Changes in Broccoli (*Brassica oleracea* L. Var. *italica*) Health-Promoting Compounds with Inflorescence Development

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Changes in phenolic compounds, total glucosinolates, and vitamin C were monitored during the productive period along five inflorescence development stages of three broccoli commercial cultivars (Marathon, Monterrey, and Vencedor). In an attempt to identify differences due to agronomic factors, broccoli cultivars were grown under different sulfur fertilization with poor (15 kg/ha) and rich (150 kg/ha) rates. Phenolic compounds and vitamin C concentrations showed, in all broccoli cultivars, a rising trend from the first stage until the over-maturity stage, both for rich and poor sulfur fertilization. Significant differences were detected in the first two stages between rich and poor sulfur fertilization in total glucosinolates for all broccoli cultivars, where the highest concentration was always observed in the second development stage (used as minimally processed product) during poor fertilization. With regard to the last three stages, the glucosinolate concentration in the poor sulfur fertilization started to slope down until the over-maturity stage. Where rich sulfur fertilization is concerned , the highest level was reached during the third stage (used as minimally processed product also), and after that, glucosinolate concentration decreased until the fifth stage.

KEYWORDS: Broccoli; development inflorescences stages; glucosinolates; flavonoids; hydroxycinnamoyl derivatives; vitamin C; sulfur fertilization; health-promoting; HPLC-MS

INTRODUCTION

About 600 different dietary compounds have shown anticarcinogenic activity (*1*). A diet rich in vegetables, such as broccoli, may contain indole-3-carbinol, sulforaphane, different flavonoids, vitamin C, and other compounds. These compounds play an important role in the maintenance of health and disease prevention (*2*−*3*). Thus, the isothiocyanate derivative of sulforaphane (methyl sulfinyl butane glucosinolate) isolated from broccoli has proved to be a potent inducer of phase II detoxification enzymes in mouse tissues and murine hepatoma cell cultures, which may afford protection against lung cancer and toxic electrophiles (*4*−*6*). Also, past studies from broccoli showed that effects of sulforaphane in vitro experiments with rats was more efficacious than antibiotics against the bacteria responsible for ulcers, *Helicobacter pylori*, in epithelial cells of the stomach (*7*). The contribution of dietary flavonols to improve health has been suggested to be related to their high antioxidant activity (8). Dietary vitamins C, E, and A are important in an optimal diet, due to their antioxidant and free-radical scavenging activities, which play important roles in human nutrition (9, *10*). In addition, the prevention of carcinogenic nitrosamine formation in the stomach is another protective mechanism for vitamin C (*11*).

Plant age is a major determinant of the quality and quantity of health-promoting compounds in vegetables. For example, young broccoli sprouts contain about 20-fold the glucosinolate amount of the late vegetative stage (*12*).

A significant variation in the level of glucosinolates (*13*, *14*) and phenolic compounds (*15*, *16*) biosynthesized is due to variations in environmental and agronomic factors, such as water availability (irrigation), soil composition (mineral and organic nutrients), intensity of sulfur fertilization (mainly due to different effects on phenolic enzymes), and in the case of glucosinolates, the sulfur amino acid being the basic substrate in the aliphatic glucosinolates biosynthesis (*17*).

Therefore, the purpose of the present work was to evaluate the incidence of the interaction among three different factors on type and quantity of health-promoting compounds (phenolic compounds, glucosinolates, and vitamin C) of three commercial cultivars (Marathon, Monterrey, and Vencedor) from fresh harvested broccoli inflorescences. Those factors were genetic (three cultivars), agronomic (rich and poor sulfur fertilization), and orthogenic (five development stages). Thus, this report describes phenolic compounds, glucosinolates, and vitamin C levels in a 3-fold interaction (C × S; C × F; S × F; C × S × F) among cultivar (C), development stage (S) and fertilization (F).

MATERIALS AND METHODS

Materials. Three broccoli (*Brassica oleracea* L. var. *italica*) commercial cultivars (Marathon**,** Monterrey, and Vencedor) obtained from the Centro de Investigacion y Desarrollo Agroalimentario (CIDA,

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10.1021/jf0212338 CCC: $25.00
Published on Web 05/14/2003

Table 1. Evolution of Diameter and Weight Inflorescences in Three Broccoli Cultivars, along Five Development Stages during Productive Period

development stages (Marathon)[a]	diameter (mm)	weight (g)
1	27.2 ± 3.9[b]	8.3 ± 2.8
2	41.2 ± 6.0	22.8 ± 8.6
3	97.1 ± 5.8	156.8 ± 20.0
4	136.6 ± 12.5	261.3 ± 24.0
5	171.2 ± 13.0	372.5 ± 25.0
development stages (Monterrey)	**diameter (mm)**	**weight (g)**
1	29.1 ± 3.7	9.0 ± 1.2
2	43.2 ± 3.9	26.0 ± 9.1
3	99.0 ± 6.8	166.2 ± 18.1
4	139.1 ± 13.5	280.0 ± 25.8
5	173.1 ± 14.8	391.1 ± 26.4
development stages (Vencedor)	**diameter (mm)**	**weight (g)**
1	22.2 ± 3.1	8.0 ± 2.2
2	38.2 ± 4.9	20.8 ± 8.0
3	94.3 ± 4.2	151.2 ± 17.1
4	132.0 ± 10.5	249.9 ± 23.1
5	167.3 ± 14.1	361.1 ± 27.5

[a] For description, see materials and methods. [b] Values represent the mean of three replicates per cultivar ($n = 12$) ± standard deviation.

Murcia, Spain) were studied. These cultivars have been previously selected among a group of commercial and experimental clones, due to their content in health-promoting compounds (*18*).

Sampling Times Development and Fertilization. Sowing date was February 4, 2002. During the vegetative period, seedlings were transplanted 50 days after sowing, in one splitted plot of Finca Torreblanca (South-Eastern Spain, Sucina, Murcia). Along the productive period, the first development inflorescence stage, and therefore the first harvest, was at 35 days after transplanting (DAT). Then, for example, the average weight and diameter of the little inflorescence was 8.3 gr and 27.2 mm, respectively, in Marathon cv. (**Table 1**). The successive development stages occurred at 42, 49, 56, and finally 63 DAT. The second and third inflorescence development stages are very interesting, mainly due to their uses by companies as minimally processed (fresh-cut) products. The fourth one (maturity or commercial stage) corresponded to 56 DAT (eighth week) after transplanting. Finally, the fifth one (over-maturity stage) corresponded to 63 DAT (ninth week) after transplanting, to see the final development in an old tissue of all compounds. Water and pesticides were applied according to standard cultural practices in Sucina (Murcia). The plot was located on a clay soil. Before transplanting, the soil was fertilized with 150 kg/ha supplied in the form of ammonium nitrate, and 75 kg/ha of P_2O_5 and 200 kg/ha of K_2O and sulfate were applied as calcium sulfate (13% S) in two rates, 15 (poor sulfur fertilization) and 150 kg/ha (rich sulfur fertilization). Three subplots (size 15 × 20 m) for each cultivar were used for the statistical design.

Sample Preparation. At uniform size plants, free from insect and/or mechanical damage, 24 inflorescences were randomized, selected, and transported to the laboratory, where the edible portions were cut. For analytical purposes, a total of 12 inflorescences were randomly selected, comprising three replicates of four inflorescences for each cultivar, season, and sulfur fertilization. Subsamples of 20 g from each plant per replicate were combined, weighed, frozen at −70 °C, and freeze-dried. This tissue was ground into a fine powder and stored at −20 °C for further analysis.

Extraction and Desulfation of Glucosinolates. Desulfoglucosinolate contents were determined according to Kiddle et al. (*19*). Each sample (20 μL) was analyzed on a Merck-Hitachi HPLC system (Merck-Hitachi Ltd., Tokyo, Japan) consisting of a variable UV detector set at 227 nm and a Lichosphere RP-18 column (Merck, Darmstdat, Germany) (RP-18, 25 × 0.4 cm; 5 μm particle size). The mobile phase was a mixture of water (A) and acetonitrile (B). Desulfoglucosinolates were eluted off the column in 30 min. The flow rate was 1.5 mL/min in a linear gradient, starting with 2% B to reach 20% B at 28 min and 2% B at 30 min. Extraction and desulfation procedures have been analyzed according to previously described methods(*18*).

Extraction and Determination of Phenolic Compounds. Extraction of phenolic compounds was achieved as previously described (*18*). Samples (20 μL) were analyzed on a Merck-Hitachi liquid chromatograph equipped with a pump (model L-6200) and a UV−vis detector (model L-7420). Separations were achieved on a LiChroCART column (Merck, Darmstadt, Germany, ODS-18, 25 × 0.4 cm; 5 μm particle size). The mobile phase was water/formic acid (95:5, v/v) (A) and methanol (B). The flow rate was 1 mL/min, and a linear gradient, starting with 10% B to reach 15% B at 5 min, 30% B at 20 min, 50% B at 35 min, and 90% B at 40 min, was used. Chromatograms were recorded at 320 and 360 nm. Caffeoyl-quinic acid derivatives were quantified as chlorogenic acid (5-caffeoyl-quinic acid, Sigma, St. Louis, USA), flavonoids as quercetin 3-rutinoside (Sigma St. Louis, USA), and sinapic acid and ferulic derivatives as sinapic acid (Sigma St. Louis, USA). Results were expressed as mg/kg of broccoli fresh weight.

Extraction and Determination of Vitamin C. Ascorbic acid (AA) and dehydroascorbic acid (DHAA) contents were determined, with slight modifications (*18*), according to Zapata and Dufour (*20*). HPLC analysis of vitamin C (AA + DHAA) was achieved after derivatization of DHAA into the fluorophore 3-(1,2-dihydroxyethyl)furo[3,4-b]-quinoxaline-1-one (DFQ), with 1,2-phenylenediamine dihydrochloride (OPDA). Samples (20 μL) were analyzed with a Merck-Hitachi (Tokyo, Japan) liquid chromatograph equipped with a L-4000 UV detector and a L-6000 pump. Separations of DFQ and AA were achieved on a Kromasil 100 C-18 column (25 × 0.4 cm; 5 μm particle size; Tecnokroma, Barcelona, Spain). The mobile phase was methanol/water (5:95, v/v) containing 5 mM cetrimide and 50 mM potassium dihydrogen phosphate at a pH 4.5. The flow rate was 0.9 mL/min, the detector wavelength was initially set at 348 nm, and after elution of DFQ, it was manually shifted to 261 nm for AA detection. Standard solutions, column conditioning, and derivatization procedures have been previously described (*18*).

HPLC-MS. These were performed according to previous work (*21*), using an Agilent HPLC system (Agilent Technologies, Waldbronn, Germany) equipped with a binary pump G1312A, autosampler G1313 A, photodiode array detector G1315B, controlled by Agilent software v. A.08.03 and degasser G1322A, under the same chromatographic conditions as described above for HPLC analyses.

Statistical Analysis. A completely randomized experiment design was performed. The results were submitted to a multifactorial analysis of variance (ANOVA) for the three main factors (C, S, F) and a unifactorial analysis for each main factor. Mean values compared using the least significant difference (LSD).

RESULTS

Phenolic compounds were identified by their chromatographic behavior, UV spectra, and HPLC-MS analysis (*22*) and chromatographic comparisons with authentic markers. The phenolic compound patterns of all cultivars were similar to those described by other authors (*23*, *24*). In addition, eleven glucosinolates were found in broccoli inflorescences, in agreement with previous reports (*13*, *14*, *21*). These compounds were identified by their chromatographic behavior, UV spectra, and HPLC-MS analysis (*21*) and chromatographic comparisons with authentic markers (samples supplied by R. Bennett and B. Holst, IFR, Norwich).

Changes in Flavonoids Content during Development. The total flavonoid content of the different cultivars is shown in **Table 2**. A number of flavonoids, 15−20, depending on the cultivar and development stage, were detected, and were mainly quercetin and kaempferol glycosides, in agreement with previous reports on broccoli florets (*18*, *23*). Acylated derivatives were

Table 2. Total Flavonoids and Caffeoyl-quinic Acid Derivatives (mg/kg f.w.) in Rich and Poor Fertilization along Five Development Stages[a]

cultivar	devt stages	sulfur fertn	total flavonoids	caffeoyl-quinic acid derivatives[b] 1	2	total 1–2
Marathon	1	poor	16.3	34.1	6.0	40.1
		rich	23.7	29.5	7.0	36.5
	2	poor	64.4	63.2	10.5	73.7
		rich	56.5	58.7	9.3	68.0
	3	poor	103.6	55.3	8.6	63.9
		rich	188.7	67.5	9.5	77.0
	4	poor	368.0	76.2	10.9	87.1
		rich	406.1	88.7	14.4	103.0
	5	poor	711.6	94.4	12.4	106.8
		rich	909.8	115.8	21.2	137.0
Monterrey	1	poor	22.4	20.5	11.8	32.3
		rich	41.9	35.1	34.6	69.7
	2	poor	104.3	64.0	26.7	90.7
		rich	106.1	69.9	18.4	88.3
	3	poor	479.2	100.5	26.3	126.8
		rich	353.4	96.2	34.6	130.8
	4	poor	557.5	127.9	46.7	174.6
		rich	713.0	129.5	58.3	187.8
	5	poor	1035.3	167.2	76.8	244.0
		rich	1042.7	161.9	62.7	224.6
Vencedor	1	poor	32.8	43.0	9.6	52.6
		rich	22.9	38.4	13.3	51.7
	2	poor	75.6	68.2	11.3	79.5
		rich	96.2	57.1	20.3	77.4
	3	poor	104.5	68.4	9.8	78.2
		rich	263.1	94.7	15.2	109.9
	4	poor	397.7	82.5	12.0	94.5
		rich	378.5	80.7	12.0	92.7
	5	poor	974.8	130.9	25.2	156.1
		rich	1080.3	138.5	27.6	166.1

[a] Compounds numbered according to HPLC eluction order (*22*). [b] 1 = neochlorogenic acid; 2 = chlorogenic acid.

Table 3. Results of the Analysis of Variance for Total Flavonoids and for Total and Individual Caffeoyl-quinic Acid Derivatives[a]

source of variance	total flavonoids	caffeoyl-quinic acid derivatives 1	2	total 1–2
cultivar	(15.6)[d]	(2.5)[d]	(0.5)[d]	(2.9)[d]
stage	(20.2)[d]	(3.2)[d]	(0.7)[d]	(3.7)[d]
fertilization	(12.8)[d]	(2.0)[d]	(0.4)[d]	(2.4)[d]
cultivar × stage	(34.9)[d]	(5.5)[d]	(1.2)[d]	(6.5)[d]
cultivar × fertn	(22.1)[c]	(3.5)[b]	(0.8)[c]	(4.4)[b]
stage × fertn	(28.5)[d]	(4.5)[d]	(1.0)[d]	(5.3)[d]
cultivar × stage × fertn	(49.4)[d]	(7.8)[d]	(1.7)[d]	(9.2)[d]

[a] LSD values are in parentheses. [b] $P \leq 0.05$. [c] $P \leq 0.01$. [d] $P \leq 0.001$.

also present. Flavonoid concentrations showed, in all cultivars, a clear rising trend from the first development stage until overmaturity (**Table 2**). Thus, in the first development stage, the highest value in Monterrey cv. was 42 mg/kg (representing only a 4% of the total flavonoids accumulated at the end of development) and increased until 1043 mg/kg at the last one. At the commercial maturity stage, 713 mg/kg were reached (**Table 2**). Although the individual values showed that the content was higher in rich than in poor fertilization, as a general rule, there were no significant differences between poor and rich sulfur fertilization (**Table 3**). When comparing among cultivars, there is no general rule that would predict which one is richer in total flavonoids.

Caffeoyl-quinic Acid Derivatives. The caffeoyl-quinic derivatives content of the different cultivars is shown in **Table 2**.

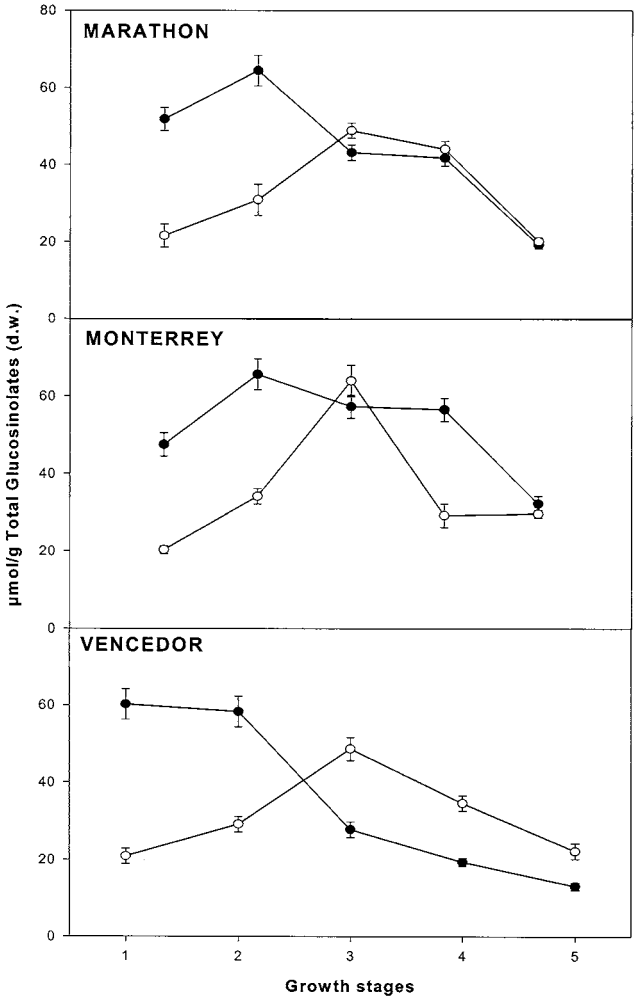

Figure 1. Evolution in total glucosinolates during five development stages of three broccoli cultivars under poor (●) and rich (○) sulfur fertilization.

Previous studies (*22*) on broccoli showed that neochlorogenic acid and chlorogenic acid were the two main caffeoyl-quinic derivatives detected. The results of this study confirm that neochlorogenic acid was the main caffeic acid derivative of broccoli. The highest value reached 167 mg/kg in Monterrey cv. in the last development stage. On the other hand, total values (**Table 2**) were close in both rich and poor fertilization, and therefore, there were no significant differences between the fertilization treatments (**Table 3**). When comparing among cultivars, Monterrey clearly showed the major amounts in total caffeoyl-quinic acid derivatives (**Table 4**). As happened in the case of flavonoids and sinapic acid derivatives, caffeoyl-quinic acid derivatives showed, in all cultivars, a clear rising trend from the first development stage until the last one (**Table 2**). Thus, in the first development stage, the highest total value in Monterrey cv. was 70 mg/kg (already representing 29% of the total caffeoyl-quinic acid derivatives at the end of development) and increased until 244 mg/kg at the last one. At maturity or commercial stage, it reached 188 mg/kg (**Table 2**).

Sinapic and Ferulic Acid Derivatives. The total content of sinapic and ferulic acid derivatives of the different cultivars is shown in **Table 4**. When comparing among individual compounds, the only sinapic and ferulic acid derivatives detected in all the cases were 1,2-disinapoylgentiobiose, 1-sinapoyl-2-feruloylgentiobiose, and 1,2-diferuloylgentiobiose (**Table 4**). Among these individual compounds, the main one was 1,2-diferuloylgentiobiose that reached 147 mg/kg in Vencedor cv.

Table 4. Total and Individual Sinapic and Ferulic Acid Derivatives (mg/kg f.w.) in Rich and Poor Fertilization along Five Development Stages[a]

cultivar	devt stages	sulfur fertn	sinapic and ferulic acid derivatives[b] 1	2	3	4	5	6	total 1–6
Marathon	1	poor	5.8	1.5	4.4	5.6	4.2	ND[c]	21.5
		rich	4.3	1.2	3.3	5.0	3.3	ND	17.1
	2	poor	12.0	3.0	3.7	10.9	12.6	10.7	59.2
		rich	10.4	3.6	13.0	11.7	11.6	7.5	57.8
	3	poor	8.5	4.3	40.5	10.0	18.7	13.2	95.2
		rich	8.3	8.2	38.2	12.5	21.5	14.2	102.8
	4	poor	14.0	7.0	31.3	9.3	18.0	11.7	91.3
		rich	13.8	13.1	48.8	14.9	29.1	14.4	134.1
	5	poor	15.7	17.4	68.1	13.4	33.9	17.5	166.0
		rich	22.6	15.1	62.7	14.1	30.2	16.5	161.2
Monterrey	1	poor	2.8	3.3	1.8	ND	ND	ND	7.9
		rich	3.5	0.8	1.5	3.8	1.8	ND	11.4
	2	poor	7.9	2.3	4.5	8.3	5.1	ND	28.1
		rich	2.1	1.8	4.7	4.8	4.2	ND	17.6
	3	poor	9.5	10.3	57.7	11.2	24.2	22.2	135.1
		rich	7.9	9.4	50.4	9.8	19.3	18.6	115.4
	4	poor	4.3	16.1	76.6	12.0	30.3	20.7	160.0
		rich	11.7	18.3	85.2	12.9	33.3	ND	161.4
	5	poor	14.1	20.4	64.7	14.7	32.6	20.4	166.9
		rich	14.7	23.5	70.4	18.3	34.2	19.3	180.3
Vencedor	1	poor	5.6	1.1	0.9	3.7	5.5	4.3	21.1
		rich	4.6	1.3	3.3	2.2	ND	ND	11.4
	2	poor	7.4	3.2	12.1	10.3	11.9	5.3	50.2
		rich	9.0	2.9	8.8	11.0	11.4	6.6	49.7
	3	poor	7.2	8.5	46.1	10.1	27.8	18.0	117.7
		rich	7.5	2.2	9.8	52.2	13.4	2.9	106.0
	4	poor	10.4	10.4	32.2	16.6	23.4	11.8	104.8
		rich	12.3	10.9	44.4	11.9	25.8	15.8	121.1
	5	poor	14.4	20.1	59.9	18.6	38.2	16.3	167.5
		rich	17.0	20.1	146.8	17.8	43.9	23.1	268.7

[a] Compounds numbered according to HPLC eluction order (*22*). [b] 1 = 1,2-disinapoylgentiobiose; 2 = 1-sinapoyl-2-feruloylgentiobiose; 3 = 1,2-diferuloylgentiobiose; 4 = 1,2,2′-trisinapoylgentiobiose; 5 = 1,2′-disinapoyl-2-feruloylgentiobiose; 6 = 1-sinapoyl-2,2′-diferuloylgentiobiose. [c] ND = not determined.

Table 5. Results of the Analysis of Variance for Total and Individual Sinapic and Ferulic Acid Derivatives[a]

source of variance	sinapic and ferulic acid derivatives 1	2	3	4	5	6	total 1–6
cultivar	(0.3)[d]	(0.2)[d]	(1.5)[d]	(0.4)[d]	(0.5)[d]	(0.3)[c]	(1.8)[d]
stage	(0.4)[d]	(0.3)[d]	(1.9)[d]	(0.5)[d]	(0.7)[d]	(0.4)[d]	(2.3)[d]
fertilization	(0.2)[d]	(0.1)[c]	(1.2)[d]	(0.3)[d]	(0.4)[b]	(0.2)[d]	(1.5)[d]
cultivar × stage	(0.7)[d]	(0.6)[d]	(3.3)[d]	(0.8)[d]	(1.1)[d]	(0.6)[d]	(4.1)[d]
cultivar × fertn	(0.4)[c]	(0.4)[d]	(2.1)[d]	(0.5)[d]	(0.7)[d]	(0.4)[d]	(2.6)[d]
stage × fertn	(0.6)[d]	(0.5)[d]	(2.7)[d]	(0.7)[d]	(0.9)[d]	(0.5)[d]	(3.3)[d]
cultivar × stage × fertn	(1.0)[d]	(0.8)[d]	(4.6)[d]	(1.2)[d]	(1.6)[d]	(0.9)[d]	(5.7)[d]

[a] LSD values are in parentheses. [b] $P \leq 0.05$. [c] $P \leq 0.01$. [d] $P \leq 0.001$.

in the last development stage. On the other hand, the total values were similar in both rich and poor fertilization, and therefore, there were no significant differences between fertilization treatments (**Table 5**). The only exception was in Vencedor cv., which, in the last development stage in rich fertilization, showed a greater value than that obtained in the poor one (**Table 4**). As in the case of flavonoids, total sinapic and ferulic acid derivative concentrations showed, in all cultivars, a clear rising trend from the first development stage until the last one. Thus, in the first development stage, the highest value was found in Vencedor cv. (21 mg/kg, representing only 8% of the total sinapic and ferulic acid derivatives at the end of development) and increased until 269 mg/kg in the last one. At commercial maturity, it reached 121 mg/kg (**Table 4**). When comparing among cultivars, similar amounts in total sinapic and ferulic acid derivatives were found. Those concentrations were close to those found for caffeic acid derivatives (especially abundant in the last development stage in all cultivars). Therefore, similar concentrations were detected among hydroxycinnamic acids. However, in this last development stage, hydroxycinnamic acids concentrations (**Tables 2** and **4**) were 4-fold lower than the total flavonoids (**Table 2**).

Glucosinolates. The aliphatic and indolic glucosinolate content of the different broccoli cultivars is shown in **Tables 6** and **7**. Previous studies (*21*) showed that 4-methylsulfinylbutyl-glucosinolate (glucoraphanin), 3-indolylmethyl-glucosinolate (glucobrassicin), and especially, 1-methoxy-3-indolylmethyl-glucosinolate (neoglucobrassicin) were the main glucosinolates in broccoli**.** In all cultivars, the content of neoglucobrassicin in both poor and rich fertilization was generally 2−4 times higher than that of glucobrassicin (the second major glucosinolate). The highest individual glucosinolate value was 55 μmol/g, corresponded to the neoglucobrassicin compound, and was found in Monterrey cv. during the second development stage with poor fertilization (**Table 7**). At commercial maturity, it reached 2, 10, and 44 μmol/g for glucoraphanin, glucobrassicin, and neoglucobrassicin (major glucosinolates), respectively (**Tables 6** and **7**). With regard to total glucosinolates (**Figure 1**), there were no significant differences between the highest value with poor fertilization (66 μmol/g in the second development stage) and the highest value with the rich one (64 μmol/g in the third development stage), both of them found in Monterrey cv. (**Tables 8** and **9**). At commercial maturity, the total glucosinolate values in all cultivars, varied between 35 and 56 μmol/g (**Table 7**).

The general behavior of glucosinolate content during the development stages was quite different from that found for phenolic compounds. Thus, as previously monitored, while phenolic compound concentrations increased along development stages and there were no significant differences between both poor and rich sulfur fertilization, total glucosinolate showed two different trends, depending on the type of fertilization. Thus, in all cultivars, values observed with the poor sulfur fertilization

Table 6. Individual Alifatic Glucosinolates (μmol/g d.w.) in Rich and Poor Fertilization along Five Development Stages[a]

cultivar	devt stages	sulfur fertn	alifatic glucosinolates[b] 1	2	3	4	5
Marathon	1	poor	0.1	0.1	0.9	ND[c]	0.2
		rich	0.3	0.2	1.0	ND	0.3
	2	poor	0.1	0.1	0.6	ND	0.5
		rich	0.3	ND	0.9	ND	0.1
	3	poor	0.1	0.1	1.3	0.1	0.1
		rich	0.1	ND	1.8	0.1	0.1
	4	poor	0.2	ND	1.3	ND	0.1
		rich	0.1	0.6	1.3	ND	0.1
	5	poor	0.1	ND	1.5	ND	ND
		rich	ND	ND	0.3	ND	0.1
Monterrey	1	poor	0.2	0.3	0.7	0.1	0.1
		rich	0.1	ND	1.3	ND	ND
	2	poor	0.9	0.2	0.8	ND	ND
		rich	0.2	0.1	1.3	ND	0.1
	3	poor	0.1	ND	1.6	0.1	ND
		rich	0.5	ND	1.4	ND	ND
	4	poor	0.1	0.9	1.9	ND	ND
		rich	0.1	0.2	1.3	ND	ND
	5	poor	0.5	0.1	1.3	ND	ND
		rich	0.1	0.1	1.6	ND	0.1
Vencedor	1	poor	0.1	0.2	1.0	0.2	0.2
		rich	0.1	ND	1.4	ND	ND
	2	poor	ND	0.1	0.6	0.1	0.3
		rich	0.2	ND	2.3	ND	0.1
	3	poor	ND	ND	1.6	0.1	ND
		rich	0.1	ND	2.0	ND	0.1
	4	poor	0.1	ND	0.9	ND	ND
		rich	0.1	0.1	0.9	0.3	ND
	5	poor	0.1	ND	0.7	ND	ND
		rich	ND	0.1	1.1	ND	ND

[a] Compounds numbered according to HPLC eluction order (*21*). [b] 1 = glucoiberin; 2 = progoitrin; 3 = glucoraphanin; 4 = glucoalyssin; 5 = gluconapin. [c] ND = not determined.

Table 7. Total and Individual Indolic Glucosinolates (μMol/g d.w.) in Rich and Poor Fertilization along Five Development Stages[a]

cultivar	devt stages	sulfur fertn	indolic glucosinolates[b] 6	7	8	9	10	11	total 1–11
Marathon	1	poor	0.5	0.1	15.1	ND	1.0	33.9	51.9
		rich	0.5	0.6	4.8	0.3	0.4	13.1	21.5
	2	poor	0.1	0.2	7.5	0.1	0.4	55.0	64.7
		rich	0.2	0.1	7.2	ND	0.5	22.0	31.0
	3	poor	0.9	0.6	15.4	0.2	0.2	24.1	43.0
		rich	0.6	0.5	11.1	ND	0.3	34.1	48.7
	4	poor	1.0	0.5	3.4	0.4	0.4	29.0	36.4
		rich	0.3	0.2	9.6	ND	0.9	30.8	43.9
	5	poor	0.5	ND	4.2	0.2	0.9	11.7	19.3
		rich	0.2	0.4	5.0	ND	0.5	13.7	20.4
Monterrey	1	poor	0.1	ND	8.4	ND	0.8	36.8	47.5
		rich	0.6	0.1	5.0	0.5	0.4	12.2	20.4
	2	poor	0.1	0.1	13.4	0.2	1.3	48.5	65.7
		rich	0.9	0.1	8.1	ND	0.7	22.6	34.0
	3	poor	0.5	0.3	4.8	0.3	1.3	48.5	57.5
		rich	0.4	ND	9.4	0.5	2.5	49.2	64.0
	4	poor	0.8	ND	7.2	0.1	1.5	43.6	56.4
		rich	0.4	ND	2.3	0.1	1.0	23.7	29.2
	5	poor	1.7	0.1	2.5	0.2	1.1	24.8	32.4
		rich	2.0	0.1	3.0	0.3	1.2	21.0	29.5
Vencedor	1	poor	0.3	0.6	14.0	ND	1.6	42.1	60.3
		rich	0.3	ND	4.8	0.2	0.6	13.4	20.9
	2	poor	0.6	0.7	14.4	ND	1.1	40.1	58.0
		rich	0.9	ND	6.4	ND	1.0	18.2	29.2
	3	poor	0.3	0.1	5.6	0.1	0.7	19.2	27.8
		rich	0.5	ND	10.8	ND	1.4	33.6	48.6
	4	poor	0.2	ND	3.7	0.2	0.3	14.0	19.6
		rich	0.1	0.1	6.7	0.5	ND	25.8	34.6
	5	poor	0.2	0.3	3.1	0.2	0.3	8.1	13.1
		rich	0.6	0.6	6.6	0.3	0.6	12.2	22.2

[a] Compounds numbered according to HPLC eluction order (*21*). [b] 6 = 4-hydroxy-3-indolylmethyl; 7 = glucobrassicanapin; 8 = glucobrassicin; 9 = gluconasturtiin; 10 = 4-methoxy-3-indolylmethyl; 11 = neoglucobrassicin.

(between 2 and 3-fold higher than those observed with rich fertilization) increased constantly during the first two development stages (reaching the maximum value during the second development stage) (**Figure 1**) and started immediately to slope down, reaching a minimal value during the fifth development stage, where the values were close to those found with rich fertilization (**Figure 1**). At commercial maturity, it reached similar values to those observed in rich fertilization, except in Monterrey cv. On the contrary, in rich sulfur fertilization, the highest level was reached during the third stage, and after that, glucosinolate concentration decreased until the fifth one. In these third and fifth development stages, Marathon and Monterrey cultivars showed no significant differences between rich and poor sulfur fertilization. Monterrey cv. presented the highest total glucosinolate levels (**Figure 1**).

Vitamin C. The vitamin C content of the different cultivars is shown in **Table 10**. As in the case of phenolic compounds, vitamin C concentrations showed, in all cultivars, a clear rising trend from the first development stage until over maturity. However, in vitamin C, the slope was lower than that obtained for phenolics, due to the large amount of vitamin C found in the first development stage. In this stage, the highest value in Marathon cv. was 81 mg/100 g (representing almost 76% of the vitamin C) and increased until 107 mg/100 g at the last one. At commercial maturity, it reached 103 mg/100 g (**Table 10**). On the other hand, as a general rule (except in Marathon cv.), there were no significant differences between poor and rich sulfur fertilization (**Table 11**). Marathon and Vencedor cultivars showed the highest values.

Table 8. Analysis of Variance for Alifatic Glucosinolates in Three Cultivars of Broccoli[a]

source of variance	alifatic glucosinolates 1	2	3	4	5
cultivar	(0.0)[e]	(0.0)[e]	(0.1)[d]	(0.0)[d]	(0.0)[e]
stage	(0.0)[e]	(0.0)[e]	(0.2)[e]	(0.0)[e]	(0.1)[e]
fertilization	(0.0)[e]	(0.0)[e]	(0.1)[e]	NS[b]	(0.0)[e]
cultivar × stage	(0.1)[e]	(0.1)[e]	(0.3)[d]	(0.1)[e]	(0.1)[d]
cultivar × fertilization	(0.0)[e]	(0.1)[e]	(0.2)[e]	(0.0)[c]	(0.1)[c]
stage × fertilization	(0.1)[e]	(0.1)[c]	(0.3)[c]	NS	(0.1)[e]
cultivar × stage × fertilization	(0.1)[e]	(0.1)[e]	(0.5)[e]	(0.1)[c]	(0.1)[e]

[a] LSD values are in parentheses. [b] NS = not significant. [c] $P \leq 0.05$. [d] $P \leq 0.01$. [e] $P \leq 0.001$.

DISCUSSION

Plant age is a major determinant of the quality and quantity of the health-promoting compound composition of plants. However, information available about the phenolic content of vegetables during different development stages is not always complete and many times is restricted to few cultivars and to a single group of phenolic compounds. To the best of our knowledge, there is no information available on the changes of Brassicas phenolic compounds and vitamin C during floret development. However, with regard to other vegetables, previous papers reported the occurrence of different flavonoid (quercetin) content in onions during development (*25*). Thus, onions grown in sandy loam soil exhibited a significant increase in total quercetin content along development stages over those harvested at the first development stage. This agrees with the results

Table 9. Analysis of Variance for the Total and Indolic Glucosinolates in Three Cultivars of Broccoli[a]

	indolic glucosinolates						total glucosinolates
source of variance	6	7	8	9	10	11	1–11
cultivar	(0.1)[e]	(0.1)[e]	(0.5)[e]	(0.1)[e]	(0.2)[e]	(0.5)[e]	(0.7)[e]
stage	(0.1)[e]	(0.1)[e]	(0.6)[e]	(0.1)[e]	(0.2)[e]	(0.7)[e]	(0.9)[e]
fertilization	(0.1)[c]	(0.1)[d]	(0.4)[e]	(0.0)[e]	(0.1)[d]	(0.4)[e]	(0.6)[e]
cultivar × stage	(0.2)[e]	(0.1)[e]	(1.1)[e]	(0.1)[e]	(0.3)[c]	(1.1)[e]	(1.6)[e]
cultivar × fertilization	(0.1)[e]	(0.1)[e]	(0.7)[c]	(0.1)[e]	NS[b]	(0.7)[e]	(1.0)[e]
stage × fertilization	(0.1)[e]	(0.1)[d]	(0.9)[e]	(0.1)[e]	(0.3)[e]	(0.9)[e]	(1.3)[e]
cultivar × stage × fertilization	(0.2)[e]	(0.2)[e]	(1.6)[e]	(0.2)[e]	(0.5)[e]	(1.6)[e]	(2.2)[e]

[a] LSD values are in brackets. [b] NS = not significant. [c] $P \leq 0.05$. [d] $P \leq 0.01$. [e] $P \leq 0.001$.

Table 10. Vitamin C (mg/100 g f.w.) in Rich and Poor Fertilization along Five Development Stages

cultivar	devt stages	sulfur fertn	vitamin C
Marathon	1	poor	59.6[a]
		rich	80.7
	2	poor	67.3
		rich	94.4
	3	poor	79.3
		rich	98.8
	4	poor	88.2
		rich	102.6
	5	poor	117.1
		rich	106.7
Monterrey	1	poor	46.6
		rich	57.1
	2	poor	57.6
		rich	65.3
	3	poor	61.7
		rich	67.3
	4	poor	64.8
		rich	86.2
	5	poor	90.7
		rich	107.6
Vencedor	1	poor	37.7
		rich	45.3
	2	poor	87.2
		rich	64.7
	3	poor	87.8
		rich	90.7
	4	poor	90.4
		rich	93.2
	5	poor	124.9
		rich	116.7

[a] Values represent the mean of three replicates per cultivar ($n = 12$).

Table 11. Results of the Analysis of Variance for Vitamin C[a]

source of variance	vitamin C[b]
cultivar	(0.4)
stage	(0.6)
fertilization	(0.4)
cultivar × stage	(1.0)
cultivar × fertilization	(0.6)
stage × fertilization	(0.8)
cultivar × stage × fertilization	(1.4)

[a] LSD values are in parentheses. [b] $P \leq 0.001$.

obtained in the present work, where broccoli flavonoid content increased along the development stages.

The phenolic content of plants depends both quantitatively and qualitatively on their genetic information. In this way, there are clear examples showing the different phenolic content of different cultivars of the same species. For example, in the case of lettuce cultivars, some of them ("iceberg" and "butter leaf" type) are very poor in flavonoids and caffeic acid derivatives, while others ("lollo rosso" and "oak leaf") contain large amounts of flavonols, caffeic acid derivatives and anthocyanins (*26*, *27*). In the case of the three broccoli cultivars analyzed in this work, similar concentrations were detected in glucosinolates, phenolics, and vitamin C.

Concerning glucosinolates, previous papers (*12*) reported that the young broccoli sprouts contain 20-fold higher amounts than the late vegetative stages. The present work has confirmed these previous studies in agreement with previous reports (*28*, *29*) that showed the highest glucosinolate levels at the start of development stage. Nevertheless, in contrast with these results, other authors (*30*) exposed that glucosinolates accumulated during the vegetative stage were catabolised during the reproductive stage. Furthermore, other authors (*31*) observed glucosinolate variation over longer periods and suggested that this variation may be due to a complex metabolic process occurring during development. Thus, during the normal development of a cruciferous plant, volatile hydrolysis products are constantly released at low concentrations, probably as a result of damage and cell death (*32*). This could explain the fall of glucosinolate concentration found in this work in proportion to broccoli inflorescence development. On the other hand, total glucosinolate concentrations showed no significant differences, in all cultivars, between poor and rich sulfur fertilization, in contrast to the results reported in *Brassicaceae* (*33*).

On the other hand, in a previous report (*34*) on tomatoes, a variation in AA content depending on ripeness was shown. It was concluded that the maturity stage can have a considerable but variable effect on AA content. Thus, Selman and Rolfe (*35*) found that the AA content of peas decreased 2-fold over the maturation period, and a 2-fold increase was observed by Rahman et al. (*36*) in green and yellow peppers. Therefore, there appears to be no clear pattern as to the effect of maturation on AA content, as concluded by Breen in his review (*37*). In our case, we concluded that the maturity stage had a considerable effect on vitamin C content in broccoli, due to the large amount observed when comparing to the initial ripening stages.

Finally, as a whole, the different behaviors during developmental stages showed among glucosinolates, phenolic compound, and vitamin C could be an interesting tool to determine the optimum harvest stage, depending on the desired compound. However, the information available on these topics is very scarce and even contradictory. Therefore, more research on these topics is needed especially in Brassicas.

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Received for review December 19, 2002. Revised manuscript received March 25, 2003. Accepted March 25, 2003.

JF0212338