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ORIGINAL PAPER

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Effect of climatic and sulphur fertilisation conditions, on phenolic compounds and vitamin C, in the inflorescences of eight broccoli cultivars

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Abstract Flavonoids, hydroxycinnamoyl derivatives (sinapic and ferulic acid derivatives+caffeoyl-quinic acid derivatives) and vitamin C were quantified by HPLC-MS in five commercial and three experimental cultivars from freshly harvested broccoli inflorescences (*Brassica oleracea* L. var. *italica*). In order to identify differences due to genetic and agronomic factors, the broccoli cultivars were grown under different climatic and agronomic conditions, i.e. early (winter) and late (spring) seasons with poor (15 kg/ha) and rich (150 kg/ha) sulphur fertilisation. The predominant sinapic and ferulic acid derivatives in all broccoli cultivars were 1,2-diferuloylgentiobiose, 1,2,2'-trisinapoylgentiobiose and 1,2'-disinapoyl-2-feruloylgentiobiose. In addition, the compounds 1,2-diferuloylgentiobiose, 1-sinapoyl-2,2'-diferuloylgentiobiose and 1,2,2'-triferuloylgentiobiose were identified in broccoli inflorescences for the first time. Extreme agronomic and environmental conditions (late season and rich sulphur fertilisation which could induce different stress situations on the plant) enhanced the phenolic content. Thus, total flavonoids showed the highest content, followed by total sinapic and feruloyl acid derivatives and total caffeoyl-quinic acid derivatives. In general, cultivars grown under rich fertilisation and late season conditions showed higher vitamin C content than those grown under the poor and early ones. Finally, results showed that commercial cultivars rendered higher amounts of phenolic compounds and vitamin C than the experimental ones.

Keywords Broccoli · Flavonoids · Hydroxycinnamoyl derivatives · Vitamin C · Harvest season

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Introduction

There has been a renewed interest in the evaluation of the phenolic content of fruits and vegetables mainly due to recent epidemiological studies. These have indicated that a diet with a high fruit and vegetable consumption has health benefits in the prevention of chronic diseases, including cardiovascular disease and certain types of cancer [1, 2]. In the last few years there has been increasing evidence of the possible role of phenolic compounds in these health benefits. Attention has been mainly paid to caffeic acid derivatives (caffeoyl-quinic acid, chlorogenic acid, etc [3, 4]) and flavonols (mainly quercetin and its derivatives [2, 5, 6]). The contribution of dietary flavonols to improved health has been demonstrated to be related to their high antioxidant activity [7]. The occurrence of at least two main flavonol glycosides (quercetin 3-*O*-sophoroside and kaempferol 3-*O*-sophoroside) in broccoli inflorescences has been reported [8].

A significant variation in the level of phenolic compounds in plant tissue may have resulted from variations in environmental and agronomic factors such as water availability (irrigation), soil composition (mineral and organic nutrients) as well as from intensity of sulphur fertilisation [9, 10], mainly due to different effects on phenolic enzymes. However, some of the topics covered in this report are sparsely covered in the literature, and when information is available, it is often old and contradictory. Rossiter and Barrow [10] showed that sulphur deficiency increased phenolic compounds concentration in clover and that a severe deficiency almost doubled the concentration of phenolics. On the other hand, light intensity and quality (wavelength) also have an important effect on phenolic metabolism as they affect flavonoid biosynthesis in general [11, 12]. Thus, Taylor [13] reported differences in phenolic compounds content between species and between seasons (daylight and photoperiods) in *Xanthium pennsylvanicum*.

Therefore, the purpose of the present work was to evaluate genetic and environmental influences on the type and quantity of total flavonoids, caffeoyl-quinic acid

derivatives and sinapic acid derivatives as well as vitamin C in the edible portions of eight genotypes of fresh harvested broccoli inflorescences (*Brassica oleracea* L. var. *italica*) including three experimental (SG-4515, I-9904, I-9809) and five commercial cultivars (Vencedor, Furia, Pentathlon, Monterrey and Marathon). All cultivars were grown under different climatic (early vs. late crop) and sulphur fertilisation (poor vs. rich) conditions. This report describes phenolic compounds and vitamin C levels in a threefold interaction among cultivar (C), season (S) and fertilisation (F) conditions, (i.e. C×S, C×F, S×F, C×S×F)in fresh harvested broccoli inflorescences.

Materials and methods

Plant material and agronomic conditions. Broccoli cultivars (*Brassica oleracea* L. var. *italica*) including five commercial and three experimental (hybrids, open-pollinated varieties, Land races) used for the assays were obtained from the Centro de Investigacion y Desarrollo Agroalimentario (Murcia, Spain). Sowing dates were 20 December 2000 and 3 March 2001 for the early and late seasons respectively. For both seasons, seedlings were transplanted 30 days after sowing in one splitted plot of Finca Torreblanca (La Alberca, Murcia, Spain). Harvest of the early season occurred between 60 and 62 days after planting. Harvest of the late season occurred between 50 and 52 days after planting. Water and pesticides were applied according to standard cultural practices in La Alberca (Murcia). The plot was placed on a clay soil. Before transplanting, the soil was fertilised with 150 kg/ha supplied in the form of ammonium nitrate, 75 kg/ha of P_2O5, and 200 kg/ha of K_2O and S were applied as calcium sulphate (13% S) at two levels, 15 kg/ha (poor sulphur fertilisation) and 150 kg/ha (rich sulphur fertilisation). Three subplots (15×20 m) for each cultivar were used for the statistical design.

Broccoli processing. At optimum maturity, 24 inflorescences were randomly selected from uniform size plants, free from insect and/or mechanical damage, and immediately 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 each cultivar, season and sulphur fertilisation. 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 determination of phenolic compounds. Extraction procedures have been analysed as previously described for phenolic compounds [14]. Samples (20 μl) were analysed 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), 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. The flow rate was 1 ml/min. Chromatograms were recorded at 320 nm and 360 nm. Caffeoyl-quinic acid derivatives were quantified as chlorogenic acid (5-caffeoylquinic acid, Sigma, St. Louis, USA), sinapic acid derivatives as sinapic acid (Sigma), and total flavonoids, as the addition of all the peak areas, as quercetin 3-rutinoside (Sigma). Results were expressed as milligrams of phenolic compound per kilogram of broccoli (fresh weight).

Extraction and determination of vitamin C. Extraction and determination of vitamin C. Ascorbic acid (AA) and dehydroascorbic acid (DHAA) contents were determined according to Zapata and Dufour [15]. HPLC analysis of vitamin C (AA+DHAA) was achieved after derivatisation of DHAA into the fluorophore 3-(1,2-dihydroxyethyl)furo[3,4-b]quinoxaline-1-one (DFQ), with 1,2-phenylenediamine dihydrochloride. Samples (20 μl) were analysed with a Merck-Hitachi (Tokyo, Japan) liquid chromatograph equipped with an L-4000 UV detector and an 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 (cetyltrimethyl ammonium bromide) and 50 mM potassium dihydrogen phosphate at 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, was manually shifted to 261 nm for AA detection. Standard solutions, column conditioning, and derivatisation procedures have been previously described [14].

HPLC-MS analysis. These analyses were performed according to previous work [16], using an Agilent HPLC system (Agilent, 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 randomised experiment design was performed. The results were submitted to a multifactorial analysis of variance for the three main factors (C, S, F) and a unifactorial analysis for each main factor. Means values were compared using the least significant difference method. Statistical analysis permitted evaluation of several effects and their interaction on the determination, and quantification of these.

Results and discussion

Hydroxycinnamoyl derivatives were identified by their chromatographic behaviour and UV spectra, HPLC-MS (Table 1) and chromatographic comparisons with authentic markers. The pattern found in broccoli cultivars (Fig. 1, Table 2, Table 3), was similar to that previously described by other authors [8, 17, 18], even if quantitative

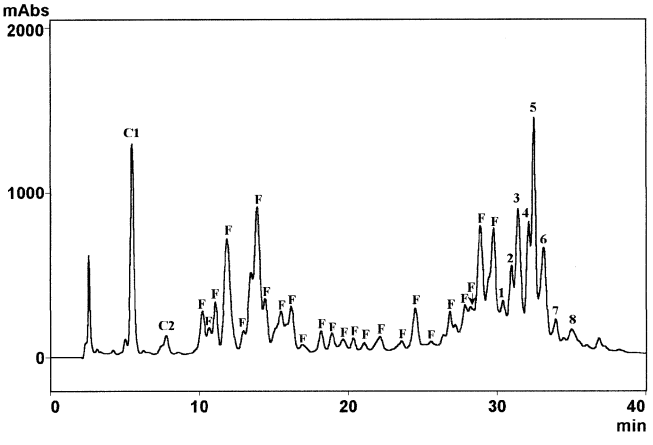

Fig. 1 HPLC chromatograms of Marathon cv. (*F*) Total flavonoids recorded at 360 nm. *C1* Neochlorogenic acid (3-*O*-caffeoyl-quinic), *C2* Chlorogenic acid (5-*O*-caffeoyl-quinic), *1* 1,2-disina-poylgentiobiose, *2* 1-sinapoyl-2-feruloylgentiobiose, *3* 1,2-diferuloylgentiobiose, *4* 1,2,2'-trisinapoylgentiobiose, *5* 1,2'-disinapoyl-2-feruloylgentiobiose, *6* 1-sinapoyl-2,2'-diferuloylgentiobiose, *7* 1,2,2'-trisinapoylgentiobiose, *8* 1,2,2'-triferuloylgentiobiose, recorded at 320 nm

Table 1 HPLC-DAD-MS analysis of broccoli inflorescences phenolics. *sh* Spectrum shoulder

	Phenolic	No.	Rt min	HPLC-DAD nm	HPLC-MS *m / z*
Caffeoyl-quinic derivatives	Neochlorogenic acid	C1	5.2	332, 295sh	353, 179
	Chlorogenic acid	C2	7.2	332, 295sh	353, 179
Sinapic acid derivatives	1,2-Disinapoylgentiobiose	1	29.2	328	753, 529, 223
	1-Sinapoyl-2-feruloylgentiobiose	2	30.0	328, 295sh	723, 499, 223
	1,2,2'-Trisinapoylgentiobiose [a]	4	30.7	328	959, 735
	1,2'-disinapoyl-2-feruloylgentiobiose	5	31.4	328, 295sh	929, 705
	1-sinapoyl-2,2'-diferuloylgentiobiose	6	32.3	320, 290sh	899, 705
	1,2,2'-trisinapoylgentiobiose [a]	7	33.7	328	959, 735
Ferulic acid derivatives	1,2-diferuloylgentiobiose	3	30.4	328, 290sh	693, 499, 175
	1,2,2'-triferuloylgentiobiose	8	36.0	320, 290sh	849

Table 2 Total flavonoids and caffeoyl-quinic derivatives levels (milligrams/kilogram f.w.) in the rich and poor fertilisations of the eight broccoli cultivars in early and late seasons. Flavonoids as rutin, and caffeoyl-quinic acid derivatives as chlorogenic acid at 320 nm

Cultivar	Season	Sulphur fertilisation	Total Flavonoids[a]	Caffeoyl-quinic derivatives[b]		Total 1–2
				1	2	
Marathon	Early	Poor	160.1	33.0	10.2	43.2
		Rich	288.6	50.6	15.4	66.0
	Late	Poor	446.0	74.8	22.3	97.1
		Rich	489.7	73.6	24.1	97.7
Furia	Early	Poor	113.2	31.4	9.8	41.2
		Rich	263.4	43.6	14.2	57.8
	Late	Poor	457.1	72.8	20.1	92.9
		Rich	604.5	87.5	25.9	113.4
Monterrey	Early	Poor	405.0	62.8	20.7	83.5
		Rich	316.8	60.4	21.1	81.5
	Late	Poor	609.7	117.7	33.2	150.9
		Rich	339.1	66.4	20.4	86.8
Pentathlon	Early	Poor	295.6	32.2	11.0	43.2
		Rich	253.2	43.8	13.4	57.8
	Late	Poor	446.1	72.1	24.2	96.3
		Rich	452.1	64.6	21.9	86.5
Vencedor	Early	Poor	290.4	28.2	10.4	38.6
		Rich	259.3	58.2	15.7	73.9
	Late	Poor	400.3	57.6	16.2	73.8
		Rich	970.1	107.9	29.7	137.6
I-9809 ×	Early	Poor	124.7	19.0	4.5	23.5
		Rich	272.3	35.6	9.8	45.4
	Late	Poor	389.0	41.7	12.1	53.8
		Rich	443.4	57.1	16.5	73.6
I-9904 ×	Early	Poor	154.4	32.7	11.1	43.8
		Rich	116.6	61.4	19.6	81.0
	Late	Poor	329.9	64.3	20.6	84.9
		Rich	591.5	73.3	22.3	95.6
SG-4515 ×	Early	Poor	68.1	24.8	9.7	34.5
		Rich	102.8	63.4	17.6	81.0
	Late	Poor	327.8	80.9	24.9	105.8
		Rich	627.7	84.4	26.3	110.7

[a] Values represent the mean of three replicates per cultivar (n=12)
[b] For compounds identification see Table 1
[c] Experimental cultivars

differences were found for the different cultivars and treatments. Thus, hydroxycinnamic acid compounds, found in lower amounts, identified as 1,2,2'-trisinapoylgentiobiose and 1,2,2'-triferuloylgentiobiose (compounds 7 and 8 respectively, Fig. 1, Table 2, Table 3), were not detected in some cultivars with certain treatments. However, the rest of hydroxycinnamic acid derivatives were common to all cultivars, season and fertilisations. In addition, as far as we are aware, 1-sinapoyl-2,2'-diferuloylgentiobiose, 1,2-diferuloylgentiobiose and 1,2,2'-triferuloylgentiobiose were identified in broccoli inflorescences for the first time.

Moreover, a number of individual flavonoids (15–20, depending on the cultivar, season and fertilisation) were detected but not fully identified (currently under study). These were mainly quercetin and kaempferol glycosides,

Table 3 Analysis of variance for the total flavonoids and caffeoyl-quinic acid derivatives in eight broccoli cultivars. LSD values are in brackets

Source of variance	Total Flavonoids	Caffeoyl-quinic derivatives		Total 1–2
		1	2	
Cultivar	(2.1)*	(1.0)*	(0.4)*	(1.6)*
Season	(1.1)*	(0.5)*	(0.2)*	(0.8)*
Fertilisation	(1.1)*	(0.5)*	(0.2)*	(0.8)*
Cultivar×season	(3.0)*	(1.3)*	(0.6)*	(2.0)*
Cultivar×fertilisation	(3.0)*	(1.3)*	(0.6)*	(2.0)*
Season×fertilisation	(1.5)*	(0.6)*	(0.3)*	(1.2)*
Cultivar×season×fertilisation	(4.2)*	(2.0)*	(0.9)*	(3.0)*

* $P \leq 0.001$

Table 4 Average maximum and minimum air temperatures, sunlight, and rainfall from January to July for La Alberca area

Season	Average air temp ºC		Sunlight h	Rainfall mm
	Min.	Max.		
January-April[a]	7.8	18.8	264.0	20.5
April-July[b]	14.9	25.9	348.8	5.4

[a] Early
[b] Late

in agreement with previous reports on broccoli florets [8, 14]. Other acylated derivatives were also present.

It is also remarkable that, in some cases, total and individual flavonoids, hydroxycinnamic acid derivatives and vitamin C were found with no significant differences in multifactorial analysis among cultivar, season and fertilisation. However, the general tendency showed that there were more phenolic compounds and vitamin C in the late season than in the early and in rich fertilisation than in poor.

Variation of flavonoids

The total flavonoid content of the different broccoli cultivars is shown in Table 2. Of the cultivars, Vencedor and Monterrey showed the highest contents of these compounds while I-9904 and SG-4515 showed the lowest concentrations. The Vencedor cultivar reached the highest value (970.1 mg/kg f.w.) of total flavonoids in the late season and rich fertilisation. This value was threefold higher than that previously reported by Price et al. [8]. In contrast, SG-4515 contained only 68.1 mg/kg f.w. in the early season in poor fertilisation (Table 2). Total flavonoids levels were significantly affected by cultivar, season, fertilisation and all interactions (Table 3).

These results are in agreement with those previously reported by Gil et al. [11] and Dussi [12] who showed that sun irradiation had some effects on phenolic metabolism, as it affected flavonoid biosynthesis in general. Further, according to Tso et al. [19] and Taylor [13] there was a good correlation between higher levels of flavonoids and longer light treatments. In fact, late season had more sunlight (349 h) than early (264 h), (Table 4) which could explain the significant differences found between early and late season with a higher amount of total flavonoids for the late one.

In all cases (except Monterrey) the amount of total flavonoids with rich fertilisation was significantly larger than with the poor (about two- to threefold, Table 2). Previous studies have shown that sulphur deficiency increases clover phenolics [10], in contrast to our results, which confirmed a big increase in total flavonoids for rich fertilisation versus poor fertilisation in all broccoli cultivars. An explanation for the large amount of total flavonoids, found here could be the extreme agronomic and environmental conditions (with a high temperature in late season and a high sulphur fertilisation dosage) to which broccoli was exposed. This led to the inhibition of both phenolic enzymes polyphenol oxidase (PPO) and peroxidase (POD) because of the abiotic stress, as previously reported by Tomás-Barberán and Espín [20]. More research on these topics is needed, specifically the different effects on phenolic compounds and phenolic enzymes (phenyl amonio lyase, POD and PPO) depending on factors such as agronomic practices.

Variation of hydroxycinnamoyl derivatives

The hydroxycinnamoyl derivatives contents of the different cultivars are shown in Table 2 and Table 5. Among the cultivars, Monterrey was the richest in these compounds and reached the highest total sinapic and feruloyl acid derivatives value, containing 201.4 mg/kg f.w., similar to that previously reported by other authors [18, 21], in which broccoli inflorescences contained several mixed feruloylsinapoyl esters of gentiobiose. Regarding caffeoyl-quinic derivatives, these were found in large amounts, reaching values close to those found in total sinapic derivatives (Table 2). Thus, Monterey cv. presented the highest total caffeoyl-quinic derivatives value (150.9 mg/kg f.w., Table 2), sevenfold higher than previously reported by other authors [18, 21]. On the other hand, SG-4515 and I-9809 were the cultivars that contained the lowest values of sinapic and caffeoyl-quinic derivatives respectively (Table 2 and Table 5).

When analysing individual hydroxycinnamoyl derivative levels it could be seen that these were significantly affected by cultivar, season, fertilisation and all interactions (Table 3, Table 6). The main sinapic and feruloyl acid derivatives found in all cultivars were 1,2-diferu-

Table 5 Total and individual sinapic and ferulic derivatives levels (milligrams/kilogram f.w.) in the rich and poor fertilisations of the eight broccoli cultivars in early and late seasons. Sinapic and feruloyl derivatives at 320 nm. Values represent the mean of three replicates per cultivar (n =12)

Cultivar	Season	Sulphur fertilisation	Sinapic and ferulic derivatives[a]								Total 1–8
			1	2	3	4	5	6	7	8	
Marathon	Early	Poor	10.9	5.0	7.8	24.3	12.3	15.2	4.8	0.0	62.6
		Rich	16.7	34.1	16.0	0.9	1.9	1.0	5.7	0.5	76.9
	Late	Poor	10.2	16.4	29.0	23.0	36.2	21.3	4.8	1.4	142.3
		Rich	7.0	14.8	29.1	22.2	33.6	21.0	5.0	3.8	136.5
Furia	Early	Poor	6.5	10.9	14.9	11.2	12.2	2.3	4.7	0.0	68.7
		Rich	25.0	14.3	22.6	8.9	1.5	0.9	1.1	1.2	75.5
	Late	Poor	10.8	15.2	28.3	25.4	38.1	21.4	4.5	1.4	145.2
		Rich	34.2	30.4	53.0	30.3	6.5	1.6	7.3	1.2	164.6
Monterrey	Early	Poor	7.7	22.7	14.8	26.4	10.4	18.5	4.3	0.0	104.7
		Rich	9.8	28.3	15.2	22.5	10.7	0.8	0.8	6.1	94.2
	Late	Poor	49.9	36.0	59.8	39.7	6.3	2.1	6.3	1.4	201.4
		Rich	3.8	2.4	8.7	29.6	16.6	34.0	22.1	7.2	124.3
Pentathlon	Early	Poor	5.2	6.0	13.8	16.5	14.6	9.4	3.1	0.0	78.1
		Rich	1.9	11.0	27.9	18.8	27.5	11.6	1.0	4.3	104.1
	Late	Poor	29.4	10.1	17.2	28.1	26.9	39.6	22.2	4.9	178.5
		Rich	7.0	13.2	24.0	21.4	35.8	20.1	4.1	3.4	129.0
Vencedor	Early	Poor	8.4	5.1	11.3	13.3	7.2	10.0	3.1	9.9	68.4
		Rich	11.8	2.1	8.6	20.5	13.7	23.0	9.3	0.0	89.0
	Late	Poor	5.0	13.5	18.8	27.7	29.3	10.3	0.5	1.1	106.2
		Rich	25.5	51.1	32.7	40.8	9.5	2.4	8.8	2.2	173.0
I-9809 [b]	Early	Poor	5.5	7.5	11.4	15.8	15.2	14.3	0.0	0.0	69.7
		Rich	27.1	23.8	27.8	11.3	1.2	1.1	2.5	1.8	96.6
	Late	Poor	15.1	31.8	29.8	38.7	16.4	1.5	1.5	1.9	136.7
		Rich	6.8	10.9	36.5	20.3	39.5	17.3	2.7	4.4	138.5
I-9904 [b]	Early	Poor	10.7	1.7	4.1	7.3	8.4	15.5	16.8	13.6	78.1
		Rich	14.2	18.9	20.8	21.8	1.8	3.5	1.6	1.3	83.8
	Late	Poor	2.7	2.3	16.4	22.8	21.5	22.7	8.4	1.7	98.6
		Rich	20.6	32.7	26.6	34.4	12.7	0.7	4.1	0.5	132.3
SG-4515 [b]	Early	Poor	4.1	3.3	5.5	13.8	12.4	9.8	6.2	2.2	57.3
		Rich	39.0	19.9	26.5	10.3	0.9	0.6	1.5	2.2	100.8
	Late	Poor	27.2	6.5	16.6	32.0	25.5	36.7	15.5	1.4	161.6
		Rich	9.9	20.6	41.9	24.6	42.0	23.3	4.5	5.1	171.9

[a] For compound identification see Table 1
[b] Experimental cultivars

Table 6 Analysis of variance for the total and individual sinapic and ferulic acid derivatives in eight broccoli cultivars. LSD values are in brackets. *NS* Not significant

Source of variance	Sinapic and ferulic derivatives								Total 1–8
	1	2	3	4	5	6	7	8	
Cultivar	(0.6)*	(0.6)*	(0.7)*	(0.7)*	(0.7)*	(0.6)*	(0.4)*	(0.3)*	(1.5)*
Season	(0.3)*	(0.3)*	(0.3)*	(0.3)*	(0.3)*	(0.3)*	(0.2)*	NS	(0.7)*
Fertilisation	(0.3)*	(0.3)*	(0.3)*	(0.3)*	(0.3)*	(0.3)*	(0.2)*	(0.1)*	(0.7)*
Cultivar × season	(0.8)*	(0.8)*	(0.9)*	(0.9)*	(0.9)*	(0.8)*	(0.6)*	(0.4)*	(2.1)*
Cultivar × fertilisation	(0.8)*	(0.8)*	(0.9)*	(0.9)*	(0.9)*	(0.8)*	(0.6)*	(0.4)*	(2.1)*
Season × fertilisation	(0.4)*	(0.4)*	(0.4)*	NS	(0.4)*	(0.4)*	(0.3)*	(0.2)*	(1.1)*
Cultivar × season × fertilisation	(1.1)*	(1.1)*	(1.3)*	(1.3)*	(1.3)*	(1.1)*	(0.9)*	(0.6)*	(3.0)*

* $P \leq 0.001$

loylgentiobiose, 1,2,2'-trisinapoylgentiobiose and 1,2'-disinapoyl-2-feruloylgentiobiose (representing values between 23% and 32% of total sinapic and feruloyl acid derivatives in all cultivars). In the present work, the highest individual sinapic and ferulic acid derivative was found for 1,2-diferuloylgentiobiose (59.8 mg/kg f.w.), in poor fertilisation and late season for Monterrey cv. (Table 5). With regard to caffeoyl-quinic derivatives, the highest value was the compound identified as neochlorogenic acid (117.7 mg/kg f.w.) with poor fertilisation in the late season in Monterrey cv. too (Table 2). All the individual compounds were significantly affected by cultivar, season, fertilisation and all interactions except 1,2,2'-trisinapoylgentiobiose, with no significant differ-

Table 7 Vitamin C levels (milligrams/100 g f.w.) in the rich and poor fertilisation of the eight broccoli cultivars in early and late seasons. Values represent the mean of three replicates per cultivar (n =12)

Cultivar	Season	Sulphur fertilisation	Ascorbic acid	Dehydroascorbic acid	Vitamin C
Marathon	Early	Poor	62.3	16.2	78.5
		Rich	57.4	20.5	77.9
	Late	Poor	68.4	23.2	91.6
		Rich	82.9	24.6	107.5
Furia	Early	Poor	65.9	24.1	90.0
		Rich	34.3	29.7	64.1
	Late	Poor	67.0	24.1	91.2
		Rich	76.2	36.1	112.3
Monterrey	Early	Poor	53.0	19.2	72.2
		Rich	61.9	25.0	86.9
	Late	Poor	72.3	31.1	103.4
		Rich	68.5	36.8	105.3
Pentathlon	Early	Poor	69.8	23.2	93.0
		Rich	72.4	25.6	98.0
	Late	Poor	89.9	30.0	119.9
		Rich	91.9	27.3	119.1
Vencedor	Early	Poor	73.4	27.5	100.9
		Rich	54.0	18.6	72.7
	Late	Poor	91.1	31.5	122.6
		Rich	75.9	24.4	100.3
I-9809 [a]	Early	Poor	73.5	18.3	91.8
		Rich	77.3	20.5	97.8
	Late	Poor	85.3	20.1	105.5
		Rich	81.9	21.7	103.6
I-9904 [a]	Early	Poor	51.6	35.2	86.8
		Rich	63.9	27.1	91.0
	Late	Poor	74.5	24.4	98.9
		Rich	62.6	18.2	80.8
SG-4515 [a]	Early	Poor	54.6	21.2	75.7
		Rich	56.4	18.6	75.0
	Late	Poor	87.8	33.8	121.7
		Rich	86.4	30.0	116.4

[a] Experimental cultivar

Table 8 Analysis of variance for the vitamin C in eight broccoli cultivars. LSD values

Source of variance	Ascorbic acid	Dehydroascorbic acid	Vitamin C
Cultivar	(1.9)*	(1.0)*	(2.6)*
Season	(0.9)*	(0.5)*	(1.3)*
Fertilisation	(0.9)*	NS	(1.3)*
Cultivar×season	(2.6)*	(1.4)*	(3.7)*
Cultivar×fertilisation	(2.6)*	(1.4)*	(3.7)*
Season×fertilisation	(1.3)*	NS	NS
cultivar×season×fertilisation	(3.7)*	(2.0)*	(5.3)*

* $P \leq 0.001$

ences for S×F interaction (Table 6). Another exception was found for 1,2,2'-triferuloylgentiobiose, with no significant differences for season (Table 6).

Vitamin C content

Vitamin C levels were significantly affected by cultivar, season, fertilisation and all interactions except for S×F (Table 7, Table 8). Therefore, no significant differences were noted in vitamin C when comparing different fertilisation at both growing seasons. These levels were also irregular, ranging from 64.1 mg/100 g f.w. in Furia to 121.7 mg/100 g f.w. in SG-4515 (Table 7). It is remarkable that this last amount is higher than that previously reported by other authors [22, 23]. Results showed that commercial varieties rendered higher amounts of vitamin C than experimental cultivars (similar to what was observed for phenolic compounds). However, the highest amount was found in SG-4515, which reached a value of 121.7 mg/100 g f.w. (Table 7). As a general rule, cultivars grown under rich fertilisation conditions showed no significant differences to those grown under the poor one. However, in general, regarding the season, those cultivars grown in the late season shower higher vitamin C contents than those grown in the early one.

In conclusion, the results obtained in this work showed the significant influence of the sulphur fertilisation on the amount of flavonoids and hydroxycinnamic acid derivatives. Thus, in general, rich fertilisation and late season showed higher concentrations than poor fertilisation and early season. On the contrary, in the case of vitamin C, cultivars in general presented no significant differences of these compounds between rich and poor sulphur fertilisation. However, in general, regarding the season, those cultivars grown in the late season shower higher vitamin C content than those grown in the early one. Also, the large amount of total flavonoids compounds quantified here, in comparison with what has been previously reported for broccoli inflorescences is remarkable. Moreover, Monterrey and Vencedor were the most interesting commercial cultivars due to their large amounts of phenolics and vitamin C. On the other hand, SG-4515 was the only experimental cultivar with high concentrations of total hydroxycinnamoyl acid derivatives. The wide range of variability in phenolics, among and within the eight cultivars that were evaluated in this study, offer an important base for developing cultivars with enhanced health benefits. ,It should be noted that the proper identification of new compounds in broccoli will increase a little more the interest in this well-studied vegetable.

Finally, more research is needed for the final explanation of the relationship among environment, agronomic practices and phenolic enzymes in broccoli inflorescences.

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