THE Society for Food Science & Technology
Journal of Food Science
A Publication of the Institute of Food Technologists

Broccoli-Derived By-Products—A Promising Source of Bioactive Ingredients

RAÚL DOMÍNGUEZ-PERLES, MARIA CARMEN MARTÍNEZ-BALLESTA, MICAELA CARVAJAL, CRISTINA GARCÍA-VIGUERA, AND DIEGO A. MORENO

ABSTRACT: The regular dietary intake of broccoli on a weekly basis has been related to better health, but industrial use of broccoli by-products (crop remains) is negligible. Adding value to broccoli by-products in a country such as Spain, one of the main broccoli producers for the EU, is of scientific and economic interest. The present article is focused on the bioactive compounds (glucosinolates, phenolic acids, and flavonoids) and nutrients (vitamin C, minerals, and trace elements), as well as the *in vitro* radical-scavenging capacity (DPPH· test), of the broccoli products (harvest remains) resulting from greenhouse cultivation using 80 mM NaCl treatment, representative of the currently available irrigation water in the production areas of Murcia (SE Spain). The bioactive compounds and nutrient contents varied according to the cultivar, organ (leaves or stalks), and the saline stress (80 mM NaCl), in the cultivars Marathon, Nubia, and Viola. Cultivar Nubia was not affected dramatically by 80 mM NaCl and the contents of phytochemicals and nutrients in the by-products of Nubia fell within the range of health-promoting levels of edible commercial parts (inflorescences or flower heads). Therefore, adding value to broccoli agrowaste by obtaining bioactive ingredients and nutrients could benefit the food and drug industry.

Practical Application: Many by-products of the agrifood industry may be useful as sources of nutrients and potentially functional ingredients, giving the opportunity to obtain added-value products. Previous studies have been focused on edible florets, but in this case we are interested in adding value to broccoli by-products that represent a real problem in the production sites because no intended use for this material has been envisaged. Therefore, the aim of this study was to add value to the broccoli-derived by-products, since recycling all this agrowaste to obtain bioactive ingredients for industry can boost profits and reduce costs and environmental problems.

Keywords: antioxidant activity, bioactive, *Brassica*, by-products, chemical composition

Introduction

Broccoli (*Brassica oleracea* var. *italica)* has acquired a considerable relevance in the last few years as a health-promoting food. The healthy food attributes assigned to broccoli are a consequence of its high contents of bioactive phytochemicals such as nitrogen-sulfur compounds (glucosinolates and isothiocyanates), phenolic compounds (chlorogenic and sinapic acid derivates and flavonoids) and nutrients (vitamin C, E, A, K, and so on, and essential minerals: N, K, Ca, Fe, and so on), the consumption of which is beneficial for the prevention of chronic disorders, such as carcinogenic and cardiovascular pathologies (Premier 2002; Jeffery and others 2003; Moreno and others 2006). The broccoli trade represents a major socioeconomical activity in Murcia (SE Spain), where exports surpassed 64000 mT of broccoli in 2007/2008 (Proexport 2008).

Many by-products of the agrifood industry may be useful as source of nutrients and potentially functional ingredients, giving the opportunity to obtain added value products. There is then a necessity to first study the composition of every by-product and its potential for future use. Broccoli marketable florets (flower heads) represent only a minor part of the total above-ground plant biomass (< 25% of total yield; Fink and others 1999), generating a vast amount of crop remains. Moreover, the sometimes abnormally high temperatures in the winter and spring seasons of SE Spain may induce premature flowering, resulting in the total loss of the marketable yield (heads), and converting all the biomass into an unprofitable by-product (López-Berenguer and others 2006; Moreno and others 2008). This constitutes a great amount of waste, with a negative effect on the agricultural environment. Currently, the use of broccoli by-products is restricted to flour and fiber (Campas-Baypoli and others 2009) and glucosinolate standard extraction or characterization (West and others 2004; Campas-Baypoli and others 2010). The potential use of vegetable by-products as a source of bioactive compounds is getting the attention of the scientific community (Mahro and Timm 2007).

Previous studies have been focused on edible florets, but we are interested in adding value to broccoli by-products that represent a real problem at production sites because no intended use for this material has been envisaged. Therefore, the aim of this study was to add value to the broccoli-derived by-products, since recycling all this agrowaste to obtain bioactive ingredients for industry can improve profitability and reduce costs and environmental problems. To pursue this aim, we studied the different responses of Nubia—now widely used in the study area—and Viola—a newly introduced early, purple sprouting variety—broccolis in comparison with Marathon—almost unused today. The plants were all grown hydroponically and under NaCl stress (80 mM), characteristic of the current irrigation water conditions in Murcia (SE Spain). We also tested them with regard to *in vitro* antioxidant activity (DPPH test), to see whether broccoli-derived by-products are promising sources of bioactive compounds for the food and drug industries.

MS 20100057 Submitted 1/18/2010, Accepted 2/16/2010. *Authors Domínguez-Perles, García-Viguera, and Moreno are with CEBAS-CSIC, Dept. of Food Science and Technology, P.O. Box 164, E-30100, Espinardo, Murcia, Spain. Authors Martínez-Ballesta and Carvajal are with CEBAS-CSIC, Plant Nutrition Dept., P.O. Box 164, E-30100, Espinardo, Murcia, Spain. Direct inquiries to author Moreno (E-mail: dmoreno@cebas.csic.es).*

doi: 10.1111/j.1750-3841.2010.01606.x

Materials and Methods

Growth conditions and experimental design

Broccoli (*Brassica oleracea* (Plenck) Italica Group) cultivars Marathon, Nubia, and Viola were cultivated in the autumn–winter season (November 2007 to early March 2008), in a greenhouse of the CEBAS-CSIC "La Matanza" Experimental Farm (Santomera, Murcia, SE Spain), under a semiarid Mediterranean climate.

Seeds of Marathon and Nubia were obtained from Ramiro Arnedo SA (Murcia, Spain) and seeds of Viola Early Purple Sprouting Broccoli were obtained from Thompson & Morgan UK Ltd. (Poplar Lake, Ipswich, Suffolk, U.K.). Seeds were prehydrated with aerated, deionized water for 2 h and then spread and germinated in vermiculite trays for 2 d in the dark, at 28 °C in an incubator. They were then transferred to a growth 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) and photosynthetically active radiation (PAR) was 400 μmol/m^2/s^1, provided by a combination of fluorescent tubes (Philips TLD 36W/83, N.Y., U.S.A. and Sylvania F36W/GRO Munich, Germany) and metal halide lamps (Osram HGI.T400W, Munich, Germany). After 4 d, the seedlings were placed in 15-L containers filled with perlite and irrigated 2 times per day with half-strength Hoagland nutrient solution, which was replaced completely every week.

Broccoli plants (30 d old) were transplanted to the greenhouse (0 days after transplanting [DAT]). The experiments were conducted under a noncontrolled environment, in an aluminum-framed greenhouse with polyethylene covers and mechanical ceiling windows for passive venting. The humidity achieved in the greenhouse averaged 50%/80% (day/night) and the air temperature was 21/9 °C (Table 1). The greenhouse was vented when the temperatures exceeded 28 °C. The daily mean temperature and relative humidity were calculated from measurements taken every 10 min using dataloggers (AFORA SA, Barloworld Scientific, Murcia, Spain). A total of 72 broccoli plants were placed in a randomized design, using 12 plants per treatment and cultivar, with each plant being grown in a perlite-filled, 20-L container (40-cm dia), separate from each other, resulting in a density of 2 plants/m^2. All plants were grown under the same conditions and were irrigated with $\frac{1}{2}$ strength Hoagland nutrient solution (approximately 2 dS/m E.C.) twice a week under natural light conditions, until 20 DAT. At this point, the application of 80 mM NaCl in the nutrient solution started (approximately 7 dS/m E.C.). The plants did not show any symptom of deficiency or toxicity. Additional air temperature and relative humidity data are listed in Table 1. At 90 DAT, all the plants were harvested.

The plants of each treatment were chosen for fresh and dry weight determination. Plant fresh weight (total above-ground biomass) was recorded directly, and inflorescences were separated from the leaves and stalks (by-products) (Table 2). For analytical purposes, the sampled material was cut into small pieces and mixed thoroughly, to be bulked again into 3 well-mixed replicates per treatment and cultivar ($n = 3$). The samples were then flash-frozen using liquid nitrogen and kept at −80 °C until being freeze-dried (Christ Alpha 1-4D, Christ, Osterode am Harz, Germany), ground to a fine powder and stored at −20 °C for further analysis.

Extraction and determination of intact glucosinolates and phenolic compounds

Freeze-dried powder (50 mg) was extracted in 1.5 mL of 70% MeOH for 30 min at 70 °C, vortexed every 5 min to improve extraction and then centrifuged (20 min, 10000 × g, 4 °C) (Sigma 1-13, B. Braun Biotech Intl., Osterode, Germany). The supernatants were collected and the methanol was removed using a rotary evaporator; the dried residue was reconstituted in ultrapure water to 1 mL and filtered through a 0.2-μm inorganic membrane filter (ANOTOP 10 plus, Whatman, Maidstone, U.K.). Each sample (20 μL) was analyzed in a Waters HPLC system (Waters Cromatografía S.A., Barcelona, Spain), consisting of a W600E multisolvent delivery system, inline degasser, W717plus autosampler and W2996 PAD. The compounds were separated in a Luna C18 column (25 × 0.46 cm, 5 μm particle size; Phenomenex, Macclesfield, U.K.) with a security guard C18-ODS (4 × 30 mm) cartridge system (Phenomenex). The mobile phase was a mixture of water/trifluoroacetic acid (99.9 : 0.1, v/v) (A) and acetonitrile/trifluoroacetic acid (99.9 : 0.1, v/v) (B). The flow rate was 1 mL/min in a linear gradient, starting with 1% B for

Table 1 — Experimental conditions (day/night) for the hydroponic culture of broccoli from Dec. 2007 to March 2008, in a noncontrolled-environment greenhouse in the Mediterranean climate of SE Spain (Murcia).

	Plant age (DAT)[a]						
	0	1 to 15	16 to 30	31 to 45	46 to 60	61 to 75	76 to 90
Air temperature (°C)[b]	19.5/6.4	20.6/10.3	21.6/9.9	20.6/9.9	20.4/11.8	24.3/12.1	26.7/12.4
Relative humidity (%)[b]	50.0/91.9	48.5/73.0	50.9/81.5	50.4/81.5	64.7/91.5	35.9/68.5	43.7/75.7

[a]Days after transplanting (DAT) to the greenhouse; 30-d-old plants = 0 DAT.
[b]Average values of the time period.

Table 2 — Broccoli above-ground biomass at harvest and derived by-products.

Cultivar	Treatment (mM NaCl)	Biomass (g/plant)				By-products (%)[A]
		Total	Florets	Stalks	Leaves	
Marathon	0	743.38b[B]	51.03b	116.45a	558.29b	90.8b
	80	463.13d	8.19c	70.99b	379.25d	97.2a
Nubia	0	877.03a	89.63a	125.42a	650.16a	88.8b
	80	499.66cd	6.14c	66.11b	425.14cd	98.3a
Viola	0	797.89ab	3.62c	127.08a	659.19a	98.5a
	80	555.99c	–	81.78b	470.05c	99.3a
ANOVA P-value		***[C]	**	***	***	***
LSD ($P < 0.05$)		86.96	20.75	16.81	66.37	3.3

[A]Biomass of by-products with respect to the total aerial biomass at harvest (%).
[B]Means ($n = 3$) within a column followed by the same lowercase letter are not significantly different at $P < 0.05$ according to Duncan's multiple range test.
[C]Nonsignificant ($P > 0.05$); significant at $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***).

5 min to reach 17% B at 15 min, which was maintained for 2 min, then 25% B at 22 min, 35% B at 30 min, 50% B at 35 min, and 99% B at 40 min. The monitored compounds eluted off the column in 35 min. Glucosinolates present in the samples were then identified using a previously described LC-MS method (Martínez-Sánchez and others 2006) and quantified using sinigrin as standard (sinigrin monohydrate from *Sinapis nigra*, Phytoplan Diehm&Neuberger, Gmbh, Heidelberg, Germany) at 227 nm. The chromatograms of phenolic compounds were recorded at 330 nm and quantified using external standards: caffeoylquinic acid derivates using chlorogenic acid (Sigma-Aldrich, St. Louis, Mo., U.S.A.), flavonoids with quercetin-3-rutinoside (Sigma-Aldrich) and sinapic acid derivatives using sinapinic acid (Sigma-Aldrich).

Extraction and determination of vitamin C

The ascorbic acid and dehydroascorbic acid contents (vitamin C) were determined according to the method of Vallejo and others (2002), with further modifications (Moreno and others 2008). HPLC analysis 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 HPLC (Tokyo, Japan), equipped with an L-4000 UV detector and an L-6000 pump. Separation of DFQ and L-AA was achieved on a Lichrospher RP18 100 C_{18} column (25 $\times$ 0.4 cm; 5 μm particle size 250 $\times$ 4 mm column (Scharlab, Barcelona, Spain). The mobile phase was methanol/water (5 : 95, v/v) containing 5 mM cetrimide and 50 mM potassium dihydrogen phosphate at pH 4.5. The flow rate was 0.9 mL/min; the detector wavelength was set initially at 348 nm and, after the elution of DFQ, was shifted manually to 261 nm for L-AA detection.

DPPH test

To analyze the antioxidant capacity of broccoli extracts, we used the 2,2-diphenyl-picryl-hydrazyl (DPPH•) assay, following the method of Llorach and others (2004). Briefly, 50 mg of freeze-dried material were extracted in 70% MeOH for 5 min, using an ultrasound bath, followed by centrifugation at 10000 $\times$ *g* for 10 min at 4 °C. The free radical-scavenging activity was determined using DPPH• (Sigma). It was evaluated by measuring the variation in absorbance at 515 nm after 50 min of reaction at 25 °C. All reactions were started by adding 25 μL of the corresponding diluted sample to the cuvette containing the diluted stock solution up to absorbance 1 (975 μL, from 0.094 mM free-radical DPPH•). The final volume of the assay was 1 mL. The DPPH• reactions were followed with a spectrophotometer (V-630 Jasco, Tokyo, Japan) and results were expressed as Trolox equivalents.

Analysis of mineral elements

The analysis of P, Ca, Mg, K, Na, Fe, Mn, and Zn, carried out after HNO_3 – $HClO_4$ (2 : 1) acid digestion of the dried plant material, was performed by ICP-spectrometry (OES Thermo ICAP 6000 SERIES®; Thermo Electron Corp., Franklin, Mass., U.S.A.), diluting the extract aliquot with $LaCl_3$ + CsCl, as reported elsewhere (López-Berenguer and others 2006). The total C and N in the samples were determined in a Thermo FlashEA 1112 autoanalyser (Thermo Fisher Scientific SA, Madrid, Spain).

Statistical analysis

The data were processed using the SPSS 15.0 software package (SPSS Inc., Chicago, Ill., U.S.A.). We carried out an analysis of variance (ANOVA) and a multiple range test (Duncan's test). In addition, a correlation analysis was performed to corroborate relationships between selected parameters (at $P < 0.05$).

Results and Discussion

Biomass

Regarding the aerial biomass at harvest (Table 2), Nubia broccoli performed better than Marathon in terms of total biomass (by 18%) and floret weight (by 75%), even though, on average, the harvested heads were below the commercial size (Vallejo and others 2003). All plants exhibited a head in the center of the leaves, the size of which was decreased at 80 mM NaCl. The collection of the plants was carried out during the heading physiological process, but the abnormally high temperatures of the season (Table 1) prevented the plants from reaching the commercial/maturity stage in terms of floret size and weight. For the broccoli by-products (stalks and leaves), the same trend was found, with Nubia outperforming Marathon in terms of fresh weight at harvest (Table 2). The variety Viola performed poorly under these climatic conditions with only a reduced number of heads of very low weight and the poorest biomass, generating 99% of by-product at 80 mM NaCl.

Broccoli is a cool-season crop that may be tolerant of at least mild-high soil temperature stresses, although these temperature responses are cultivar dependent (Díaz-Pérez 2009). To some extent, Nubia surpassed Marathon in terms of total biomass and floret weight. However, the commercial stage of broccoli for the fresh market and exports (maturity) is reached when the inflorescence exceeds 130 mm in diameter and 200 g fresh weight (Vallejo and others 2003) and in the present study the plants grown hydroponically, inside a nontemperature-controlled greenhouse, had not reached this commercial size at harvest, even though the plants were harvested at heading, when they had more than 15 young, mature leaves. In addition, although Marathon and Nubia have been grown in the Mediterranean area, their performance in terms of floret development was reduced dramatically by the abnormally high temperatures in this season (Table 1) and by the negative influence of NaCl (Table 2).

Genetic (cultivar), physiological (organ), and environmental (salinity) factors can all affect the bioactive compounds in broccoli (Vallejo and others 2002; Martínez-Ballesta and others 2008; Moreno and others 2008), therefore determining its nutritive value and phytochemical composition (López-Berenguer and others 2008; Oh and others 2009). Moreover, in NaCl-affected soils, poorer crop performance is characteristic (Hernández Bastida and others 2004). Even though broccoli is considered to be a crop that is "moderately sensitive" to salinity (Shannon and others 2000; López-Berenguer and others 2006; Martínez-Ballesta and others 2008), its characteristic "biphasic response" to high-salinity treatments (80 mM NaCl) is marked by growth reductions and physiological disorders that reduce the final yield (López-Berenguer and others 2008, 2009), as can be seen in Table 2 for the 80 mM NaCl treatment. Regarding vegetative biomass, both Marathon and Nubia significantly exceeded the values of Viola, a newly introduced cultivar that is less adapted to the environmental conditions of Murcia and was the most affected by 80 mM NaCl, which limited plant and flower development (Table 2). Taking all this into account, we can recommend the use of Nubia rather than Marathon (currently underutilized in the area), based on performance and adaptation to the conditions of the production area.

Glucosinolates

The effect of the cultivar and the imposed "NaCl stress" (Munns 2002) on the total glucosinolates—the sum of the individual glucosinolates analyzed—was significant in the broccoli by-products (Table 3). The stalks were richer in glucosinolates than the leaves, in all 3 cultivars. The leaves of Viola surpassed Nubia (by 72%)

and Marathon (by 76%) in total glucosinolates, and the same was true for the stalks, the highest total content occurring in Viola, compared with Nubia (16% lower) or Marathon (37% lower). The NaCl effect was positive in Viola, with increased total glucosinolates (doubling the amounts for Nubia and Marathon), but no significant influence of NaCl was exerted on Nubia or Marathon. The Viola stalks behaved similarly, and surpassed those of Nubia by 16% and those of Marathon by 37% in terms of total glucosinolates, in the control. At 80 mM NaCl, Viola also registered a 29% higher content than Nubia and a 43% higher content than Marathon, and in the stalks the treatment with 80 mM NaCl significantly increased the total glucosinolates of Marathon and Viola (Table 3). Considering the individual aliphatic glucosinolates, the leaves and stalks of Viola showed a concentration of glucoiberin (GI) that was 3 times higher than for Nubia and Marathon (Table 4). The glucoerucin (GE) in leaves was at a lower range of concentrations than GI and GR, and increased significantly at 80 mM NaCl for Marathon, Nubia and Viola. The highest GE concentration in the stalks was found in Viola and it increased with 80 mM NaCl (Table 4). The levels of glucoraphanin (GR) were much higher in Marathon and Nubia than in Viola, especially in the stalks. The concentration of the indol-glucosinolate glucobrassicin (GB) was higher in Viola but occurred in a similar concentration range in the 3 cultivars for both leaves and stalks; it was affected significantly by 80 mM NaCl, mainly in the stalks (Table 4). The neoglucobrassicin (NGB) concentration was higher in Viola than in the organs of Nubia or Marathon, but the effect of NaCl was only significant in the stalks of Marathon and Nubia.

The total glucosinolates (5.1 to 13.6 μmol/g dw) in the by-products of Nubia, as compared with Marathon or Viola, were in the range of previously described samples of commercial broccoli inflorescences (3.0 to 17.7 μmol/g dw; Vallejo and others 2002, 2003). Leaves (all the leaves of the plant, pooled at harvest) showed significant variations among cultivars and a lesser influence of NaCl (Table 3 and 4) as a driver of the synthesis/metabolism of glucosinolates. The range of concentrations was similar to that reported for old leaves (formed 2 wk after transplantation) by López-Berenguer and others (2009), and could be more promising regarding the use of broccoli-derived by-products, which are currently underutilized or wasted, as a cheap and natural source of glucosinolates.

The close relationship between glucosinolate metabolism and the environmental growth conditions is not fully understood (Yan and Chen 2007), and the data available refer mainly to inflorescences. Of the indole glucosinolates, GB and NGB were affected more by salinity, confirming that indole glucosinolates usually are

Table 3—Total glucosinolates (mg/g dw) in broccoli by-products from different commercial cultivars grown in hydroponics and under abiotic stress.

Organ	Cultivar	Treatments (mM NaCl) 0	80	ANOVA *P*-value	LSD ($P < 0.05$)
Leaves	Marathon	2.089b**a**[A]	2.110b**a**	**n.s.**[B]	**0.219**
	Nubia	2.134b**a**	2.438b**a**	**n.s.**	**0.314**
	Viola	3.669a**b**	4.474a**a**	******[C]	**0.271**
	ANOVA *P*-value	**	***		
	LSD ($P < 0.05$)	0.536	0.442		
Stalks	Marathon	3.277b**b**	4.024b**a**	*	**0.620**
	Nubia	3.892ab**a**	4.428b**a**	**n.s.**	**0.660**
	Viola	4.497a**b**	5.728a**a**	*	**0.915**
	ANOVA *P*-value	*	*		
	LSD ($P < 0.05$)	1.007	1.118		

[A]Means ($n = 3$) within a column followed by the same lowercase letter (cultivar) or within a row (NaCl treatment, in bold letters) are not significantly different at $P < 0.05$ according to Duncan's multiple range test.
[B]Nonsignificant ($P > 0.05$).
[C]Significant at $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***).

Table 4—Aliphatic and indole glucosinolates (mg/g dw) in broccoli by-products from different commercial cultivars grown in hydroponics and under abiotic stress.

Cultivar	Organ	Treatment (mM NaCl)	Aliphatic glucosinolates[A] GI	GR	GE	Indole glucosinolates[A] GB	NGB
Marathon	Leaves	0	0.376a[B]	0.365a	0.066b	1.080a	0.165a
		80	0.494a	0.430a	0.092a	1.033a	0.138a
		ANOVA *P*-value	n.s.[C]	n.s.	*	n.s.	n.s.
		LSD ($P < 0.05$)	0.12	0.030	< 0.001	0.118	0.001
	Stalks	0	0.576a	1.801a	0.095a	0.323b	0.286b
		80	0.680a	1.937a	0.109a	0.388a	0.417a
		ANOVA *P*-value	n.s.	n.s.	n.s.	**	***
		LSD ($P < 0.05$)	0.126	0.179	0.017	< 0.001	< 0.001
Nubia	Leaves	0	0.347a	0.389b	0.060b	1.050a	0.168a
		80	0.417a	0.539a	0.074a	1.112a	0.191a
		ANOVA *P*-value	n.s.	*	*	n.s.	n.s.
		LSD ($P < 0.05$)	0.109	0.089	0.009	0.316	0.063
	Stalks	0	0.535a	1.783a	0.113a	0.357b	0.350b
		80	0.590a	1.959a	0.076b	0.487a	0.411a
		ANOVA *P*-value	n.s.	n.s.	*	**	*
		LSD ($P < 0.05$)	0.167	0.379	< 0.001	< 0.001	0.005
Viola	Leaves	0	1.636b	0.010b	0.049b	1.281b	0.526a
		80	1.842a	0.201a	0.087a	1.891a	0.511a
		ANOVA *P*-value	*	***	**	**	n.s.
		LSD ($P < 0.05$)	0.141	0.001	0.005	0.261	0.112
	Stalks	0	2.024b	0.310b	0.141b	0.280b	0.830a
		80	2.957a	0.370a	0.145a	0.343a	0.882a
		ANOVA *P*-value	*	**	*	***	n.s.
		LSD ($P < 0.05$)	0.634	< 0.001	< 0.001	0.014	0.126

[A]GI: 3-methylslfinylpropyl-glucosinolate; GR: 4-methylsulfinylbutyl-glucosinolate; GE: 4-methylthiobutyl-glucosinolate; GB: 3-indolylmethyl-glucosinolate; NGB: *N*-methoxy-3-indolylmethyl-glucosinolate.
[B]Means ($n = 3$) within a column (for every cultivar and organ) followed by the same lowercase letter are not significantly different at $P < 0.05$ according to Duncan's multiple range test.
[C]Nonsignificant ($P > 0.05$); significant at $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***).

more affected by environmental stress (Moreno and others 2006). These findings reinforce the evidence showing the influence of the cultivar on the type and content of glucosinolates in a specific organ as well as on the response to the environment, Viola, the newly introduced variety, being more sensitive to the experimental conditions. The glucosinolate composition depends, as reported here for cultivars Marathon, Nubia, and Viola, on genetic and environmental factors and their interaction (Florez and others 2009). However, the relative importance of these factors for the broccoli by-products was determined here. The data indicate that growth under adverse conditions (salt treatment) did not decrease the levels of the individual glucosinolates in the broccoli by-products for Nubia or Marathon but significantly increased the glucosinolate contents in Viola. The influence of cultivar and environmental stress as well as the physiological role of each organ, affecting concomitantly the individual glucosinolates (aliphatic or indole glucosinolates), reinforced previous findings (Fenwick and Heaney 1983; Ciska and others 2000; Vallejo and others 2003).

Table 5—Total phenolic compounds (mg/g dw) in broccoli by-products from different commercial cultivars grown in hydroponics and under abiotic stress.

Organ	Cultivar	Treatment (mM NaCl) 0	Treatment (mM NaCl) 80	ANOVA *P*-value	LSD (P <0.05)
Leaves	Marathon	135.640a**a**[A]	152.900a**a**	**n.s.**	**48.848**
	Nubia	116.550ab**a**	139.030a**a**	**n.s.**	**59.592**
	Viola	99.377b**b**	163.177a**a**	***[B]	**24.756**
	ANOVA *P*-value	*	n.s.		
	LSD ($P < 0.05$)	29.873	65.524		
Stalks	Marathon	9.780b**a**	6.700a**b**	***	**0.748**
	Nubia	8.127b**a**	7.837a**a**	**n.s.**	**1.042**
	Viola	11.740a**a**	8.980a**a**	**n.s.**	**3.419**
	ANOVA *P*-value	**	n.s.		
	LSD ($P < 0.05$)	1.202	2.478		

[A]Means ($n = 3$) within a column (organ or cultivar) or row (NaCl treatment) followed by the same lowercase letter (normal or bold font, respectively) are not significantly different at $P < 0.05$ according to Duncan's multiple range test.
[B]Nonsignificant ($P > 0.05$); significant at $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***).

Phenolic compounds

The total concentration of phenolic compounds in the broccoli leaves was almost 10 times higher than in the stalks (Table 5). The characterization by HPLC-DAD of the phenolic profile of the 3 cultivars showed a statistically significant reduction of the total-phenolic content in Nubia and Viola leaves, being 16% and 36% lower, respectively, than in Marathon. Treatment with 80 mM NaCl increased the content of total phenolics in the leaves of the 3 cultivars, but the variability between samples prevents meaningful discussion of this effect. On the other hand, the concentration of total phenolics in stalks of Viola surpassed significantly those of Nubia (by 44%) and Marathon (by 20%) in the control treatment. Exposure to 80 mM NaCl had a nonsignificant effect for Viola and Nubia, but it decreased significantly the stalk phenolics content in Marathon.

The chlorogenic acid derivatives in Nubia and Marathon leaves (Table 6) were not affected by NaCl, the concentrations being double those found in Viola. On the other hand, the analyzed flavonoids and sinapic acid derivatives were scarcely affected by salinity in Nubia or Marathon; their contents were much higher in Viola leaves and were increased significantly at 80 mM NaCl. The hydroxycinnamic acids and flavonoids in the stalks of the 3 cultivars were in a very low concentration range and were practically unaffected by NaCl.

A number of reports describe how phenolic compounds may decrease as a consequence of saline stress when the plants undergo long-term NaCl treatments (Agastian and others 2000; López-Berenguer and others 2006, 2009; Wahid and Ghazanfar 2006). In contrast, saline stress has been shown to induce an increase in the content of phenolic compounds, although these reports referred mainly to the edible parts (florets and inflorescences) (Evers 1994; Farnham and others 2004; Scheuner and others 2005). The

Table 6—Hydroxycinnamic acid derivates and flavonoids (mg/g dw) in broccoli by-products from different commercial cultivars grown in hydroponics and under abiotic stress.

Cultivar	Organ	Treatment (mM NaCl)	Chlorogenic acid derivates	Flavonoids	Sinapic acid derivates
Marathon	Leaves	0	112.44a[A]	13.35a	9.85a
		80	127.27a	13.52a	12.12a
		ANOVA *P*-value	n.s[B]	n.s.	n.s.
		LSD ($P < 0.05$)	43.98	3.65	3.92
	Stalks	0	8.63a	0.00b	1.14b
		80	4.79b	0.40a	1.50a
		ANOVA *P*-value	***	***	*
		LSD ($P < 0.05$)	0.69	< 0.02	0.35
Nubia	Leaves	0	98.52a	11.28a	10.14a
		80	118.13a	10.90a	9.99a
		ANOVA *P*-value	n.s.	n.s.	n.s.
		LSD ($P < 0.05$)	56.16	2.95	3.87
	Stalks	0	6.56a	0.11b	1.44a
		80	5.89a	0.21a	1.72a
		ANOVA *P*-value	n.s.	*	n.s.
		LSD ($P < 0.05$)	1.64	0.08	0.50
Viola	Leaves	0	42.13b	30.59b	26.65b
		80	71.31a	39.55a	52.29a
		ANOVA *P*-value	***	**	***
		LSD ($P < 0.05$)	20.11	4.64	6.33
	Stalks	0	9.66a	0.24b	1.83b
		80	5.34b	0.54a	3.09a
		ANOVA *P*-value	**	*	*
		LSD ($P < 0.05$)	1.99	0.17	1.07

[A]Means ($n = 3$) within a column (cultivar and organs) followed by the same lowercase letter are not significantly different at $P < 0.05$ according to Duncan's multiple range test.
[B]Nonsignificant ($P > 0.05$); significant at $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***).

differences among cultivars in this study were blurred under the NaCl stress, meaning that there was little variation in the phenolic profile when salinity was imposed; in contrast to what was reported for Marathon leaves by López-Berenguer and others (2009), the concentrations of total phenolics and of each class analyzed increased significantly at 80 mM NaCl in Viola while Nubia and Marathon leaves remained unaffected. López-Berenguer and others (2006, 2009) described a decreased content of phenolic acids and flavonoids in old, mature leaves as well as in young, fully expanded leaves. Therefore, the degree to which abiotic stress may influence the content of bioactive phytochemicals in broccoli is not clear. These divergences could indicate a basic effect of abiotic stress on specific phenolic compounds (that is, phenolic acids), although the magnitude of this effect could be modulated or eased according to the cultivar (that is, Viola compared with Nubia or Marathon) and the particular organ under consideration (that is, leaves).

Therefore, Nubia, a broccoli cultivar currently grown in many areas of Murcia, could represent a substitute for Marathon, whereas Viola needs future experimentation to determine its appropriate season of production in this study area. The quantitative and qualitative differences between cultivars also illustrate the influence of genetic factors on the phenolic profile of broccoli, which was modulated by NaCl stress (Podsedek 2007; Moreno and others 2008), and the potential involvement of the secondary metabolism in the salinity tolerance of broccoli (Wahid and Ghazanfar 2006). Thus, the understanding of the multi-factorial effects of stress on the bioactive compounds in foods and the selection of different cultivars adapted to the current adverse conditions will lead to improved foods of plant origin (Moreno and others 2006, 2008).

Vitamin C

The content of vitamin C (Table 7) increased significantly in leaves as follows: Marathon < Nubia < Viola. It suffered a significant reduction with the 80 mM NaCl treatment. In the stalks, the trend was the opposite, with contents decreasing as follows: Marathon > Nubia > Viola; the influence of 80 mM NaCl was negligible. The concentration of the nutrient vitamin C, an important part of the antioxidant machinery of plant tissues (Davey and others 2000), varied significantly among organs and cultivars, confirming previous data (Podsedek 2007). The influence of the genetic background (as observed for the phenolic compounds) on the vitamin C concentration and the significant reduction in leaf concentration due to application of 80 mM NaCl highlight not only the importance of the biological factors (cultivar and organ) but also the influence of environmental stress and growth conditions, as studied before (Vallejo and others 2002; Moreno and others 2008).

It has been observed that, in NaCl-sensitive species, the content of ascorbate decreases with salinity (Ben Amor and others 2005; Huang and others 2005; Lester 2006), to reduce the cell H_2O_2 level through increased ascorbate peroxidase activity (Huang and others 2005). Therefore, the variations in vitamin C levels observed in the broccoli by-products could be related more to the combination of genetic and environmental factors than to the effect of one isolated factor (Howard and others 1999; Roy and others 2009).

Table 7— Vitamin C (mg/g dw) in broccoli by-products from different commercial cultivars grown in hydroponics and under abiotic stress.

Organ	Cultivar	Treatment (mM NaCl) 0	Treatment (mM NaCl) 80	ANOVA *P*-value	LSD ($P < 0.05$)
Leaves	Marathon	1.647c**a**[A]	1.295c**b**	*	**0.160**
	Nubia	1.860b**a**	1.637b**b**	**	**0.090**
	Viola	2.295a**a**	1.927a**b**	*	**0.272**
	ANOVA *P*-value	**[B]	*		
	LSD ($P < 0.05$)	0.169	0.187		
Stalks	Marathon	3.635a**a**	3.070a**b**	*	**0.270**
	Nubia	3.350b**a**	3.096a**a**	**n.s.**	**0.353**
	Viola	2.295c**a**	2.278b**a**	**n.s.**	**0.377**
	ANOVA *P*-value	**	***		
	LSD ($P < 0.05$)	0.160	0.453		

[A]Means ($n = 3$) within a column (organs and cultivars) or row (NaCl treatment) followed by the same lowercase letter (normal or bold font, respectively), are not significantly different at $P < 0.05$ according to Duncan's multiple range test.
[B]Nonsignificant ($P > 0.05$); significant at $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***).

In vitro antioxidant activity (DPPH· test)

Marathon broccoli, used as a reference (López-Berenguer and others 2006, 2009), had higher radical-scavenging activity in leaves than in the stalks, and also higher values than the currently commercially grown Nubia (Table 8). The values in the Viola leaf extracts were the highest, compared to Marathon (higher by 43%) and Nubia (by 48%).

The DPPH· assay has been used widely to characterize the antioxidant capacity of *Brassica* spp. vegetables (Llorach and others 2004; Zhou and Yu 2006; Arbos and others 2008). The analysis of extracts of the broccoli by-products showed a higher capacity for scavenging DPPH· in leaves than in the stalks (Table 8). Nevertheless, all the values for the processed extracts should have health-promoting activity (Boivin and others 2009). Only the well-known Marathon—currently not grown because of the nonmarketable productions of the last few years (the quality and yield parameters of the heads were below the standards for export and

Table 8— Analysis of the *in vitro* antioxidant activity of broccoli by-products from different commercial cultivars grown in hydroponics and under abiotic stress.

Cultivar	Organ	Treatment (mM NaCl)	Antioxidant activity (DPPH· test) (mmoles TROLOX/g dw)
Marathon	Leaves	0	22.826a[A]
		80	24.135a
		ANOVA *P*-value	n.s.
		LSD ($P < 0.05$)	1.702
	Stalks	0	12.046a
		80	9.427b
		ANOVA *P*-value	**
		LSD ($P < 0.05$)	1.396
Nubia	Leaves	0	22.084a
		80	13.355b
		ANOVA *P*-value	***
		LSD ($P < 0.05$)	3.050
	Stalks	0	3.884b
		80	5.979a
		ANOVA *P*-value	**
		LSD ($P < 0.05$)	0.939
Viola	Leaves	0	32.646a
		80	15.668b
		ANOVA *P*-value	***
		LSD ($P < 0.05$)	1.786
	Stalks	0	7.289a
		80	9.776a
		ANOVA *P*-value	n.s.
		LSD ($P < 0.05$)	2.215

[A]Means ($n = 3$) within a column (for every cultivar and organ) followed by the same lowercase letter are not significantly different at $P < 0.05$ according to Duncan's multiple range test.
[B]Nonsignificant ($P > 0.05$); significant at $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***).

commercialization, with regard to size, appearance, weight, grain quality and size, presence of yellow grains, and so on; the reason why new cultivars are being introduced)—was less affected by the NaCl stress. There is not a clear pattern for the leaves or the stalks in terms of response to saline stress, since Nubia and Viola leaves and Marathon stalks showed reduced activity in the 80 mM NaCl treatment.

The antioxidant capacity of broccoli extracts could be attributable to their "natural antioxidants" (Heimler and others 2006; Podsedek 2007). Although vitamin C contributes to the antioxidant profile of many vegetables (Kurilich and others 2002; Eberhardt and others 2005), in broccoli by-products it was correlated inversely to the radical-scavenging activity (Figure 1). In this sense, other molecules could contribute to this radical-scavenging

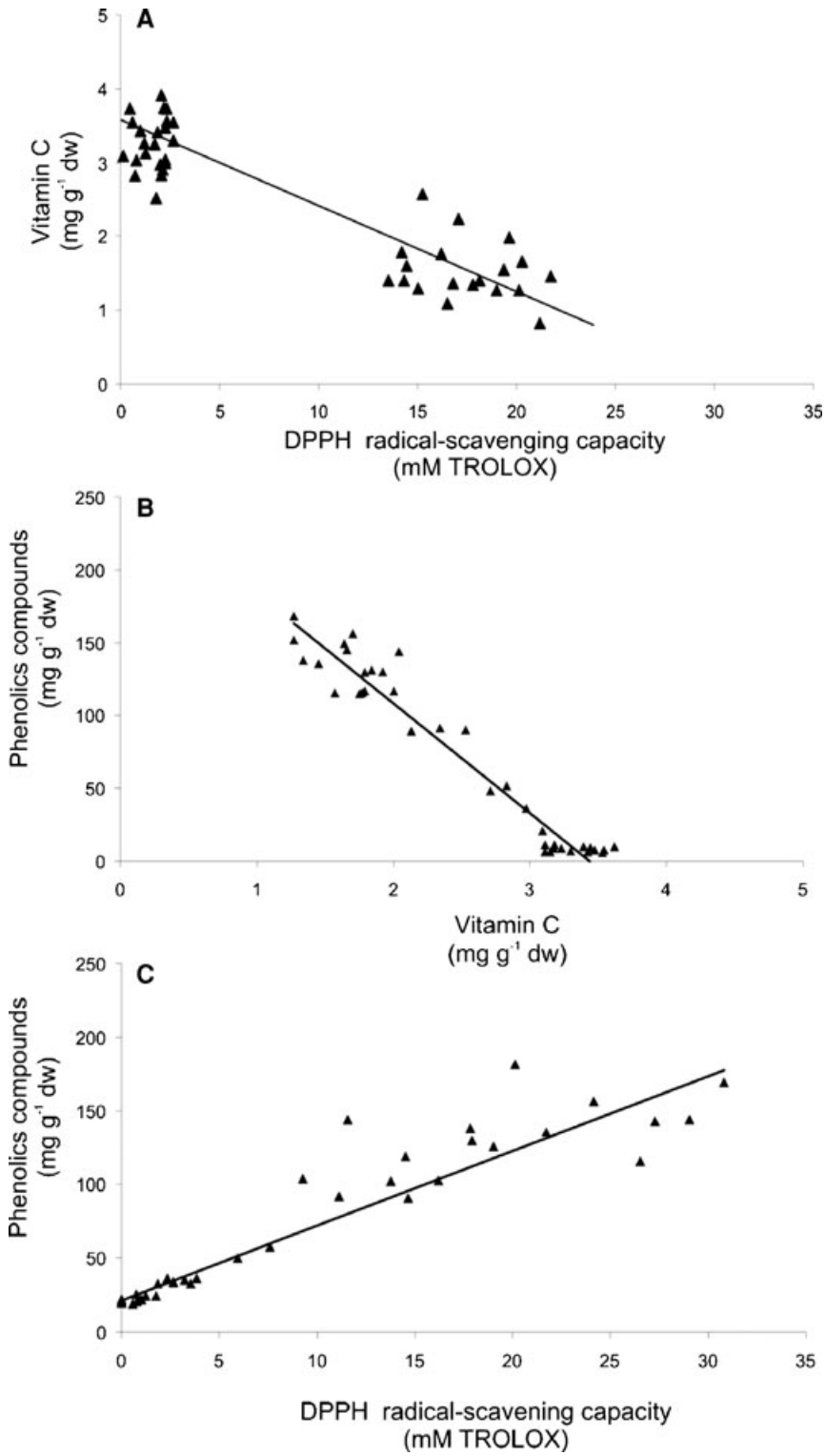

Figure 1 — Analysis of the correlation between vitamin C and DPPH· radical-scavenging activity (r^2 = 0.836) (A), phenolic compounds and vitamin C (r^2 = 0.966) (B), and phenolic compounds and DPPH· radical-scavenging activity (r^2 = 0.906) (C).

activity (as determined by HPLC-DAD), since broccoli phenolics were correlated strongly to the antioxidant DPPH· test results (Figure 1). Whereas in other studies with vegetable by-products, it was found that the level of polyphenols assessed by HPLC-DAD was not correlated significantly with the antioxidant assays (Wijngaard and others 2009); in this study, the correlation was positive and significant and this outcome supports also the potential use of broccoli-derived by-products as a good source of natural antioxidants.

Mineral nutrients and trace elements

The leaf mineral contents (Table 9) reveal that Nubia had similar concentrations to Marathon, but Viola had some quantitatively reduced amounts of minerals compared to the other cultivars (that is, total C, K, and Zn). Comparison with the USDA standard reference data revealed that the analyzed broccoli samples contained higher amounts of minerals in the leaves than those available in the database (Gebhardt and others 2009).

The total C, N, and P levels were practically unaffected by NaCl in these cultivars and fell within the sufficiency range for broccoli (Jones and others 1991).

The S levels were very similar in the 3 varieties and only were reduced significantly in Viola at 80 mM NaCl; they were almost 3 times higher than the sufficiency limit (2 mg/g dw; Jones and others 1991). Minerals such as S are essential not only for human nutrition but also for plant nutrition, and plants of *Brassica* species require high concentrations of S. The values found in broccoli leaves are similar to those found in *Brassica rapa* L. (Pekinensis group) grown under open-air cultivation and under floating plastic covers (Moreno and others 2005).

The Na in the leaves increased significantly at 80 mM NaCl, the range of concentrations being similar for the 3 cultivars. On the other hand, the K in the leaves of cultivar Viola was lower than in Nubia and Marathon. It has been reported for other plant species, and specifically for broccoli (López-Berenguer and others 2006, 2009), that the K content decreases as Na increases in plant organs, but this was not observed in our study.

The Ca content (sufficiency limit of 0.12 to 0.25 mg/g dw; Hanlon and Hochmuth 2000) was only slightly affected by NaCl in Viola. The Mg contents were in the sufficiency range (2.3 to 4.0 mg/g) for broccoli, as was found also for Fe (40 to 300 mg/kg dw). Only the Zn and Mn contents were "deficient" from the point of view of plant nutrition (sufficiency is above 45 to 90 mg/kg Zn and 25 to 150 mg/kg Mn; Hanlon and Hochmuth 2000).

The stalks (or stems) of broccoli plants were also analyzed for minerals (Table 10). The total C, N, and P in Nubia and Viola were in similar concentration ranges as in the standard Marathon and were not affected by NaCl. The total S was only increased significantly by 80 mM NaCl in Viola, and the K/Na antagonism (Jones and others

Table 9— Variation in mineral elements in the leaves of Marathon, Nubia, and Viola grown in hydroponics under abiotic stress.

		Mineral nutrients (mg/g dw)								Trace elements (mg/kg dw)		
Cultivar	Treatment (mM NaCl)	C	N	P	S	Na	K	Ca	Mg	Fe	Mn	Zn
Marathon	0	25.02a[A]	38.05a	4.60a	7.54a	1.81b	47.31a	5.47a	3.99a	107.3a	11.5b	12.3a
	80	28.02a	38.26a	4.45a	7.47a	3.92a	46.63a	5.44a	3.94a	93.8b	12.9a	12.1a
	ANOVA *P*-value	n.s.[B]	n.s.	n.s.	n.s.	***	n.s.	n.s.	n.s.	**	**	n.s.
	LSD ($P < 0.05$)	4.41	0.34	0.20	0.20	0.20	1.40	0.20	0.20	3.7	0.5	0.9
Nubia	0	25.91a	38.16b	4.44a	7.47a	1.64b	46.52a	5.75a	3.99a	89.7a	10.6b	12.0a
	80	28.46a	38.58a	4.42a	7.17a	3.83a	45.36a	5.72a	4.06a	109.9a	12.6a	11.5a
	ANOVA *P*-value	n.s.	*	n.s.	n.s.	***	n.s.	n.s.	n.s.	n.s.	**	n.s.
	LSD ($P < 0.05$)	5.06	0.34	0.20	0.32	0.20	1.87	0.20	0.28	32.5	0.63	0.89
Viola	0	18.21a	39.11a	4.15a	7.08a	1.96b	33.75a	5.42b	3.50b	87.2a	11.0b	7.5a
	80	18.06a	38.84a	4.17a	6.34b	4.29a	33.62a	6.06a	3.96a	91.5a	12.4a	8.1a
	ANOVA *P*-value	n.s.	n.s.	n.s.	*	***	n.s.	*	**	n.s.	**	n.s.
	LSD ($P < 0.05$)	1.61	0.46	0.20	0.40	0.20	0.89	0.35	0.20	10.4	0.63	1.90

[A]Means ($n = 3$) within a column (per cultivar and NaCl treatment) followed by the same lowercase letter are not significantly different at $P < 0.05$ according to Duncan's multiple range test.
[B]Nonsignificant ($P > 0.05$); significant at $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***).

Table 10— Variation in mineral elements in the stalks of Marathon, Nubia, and Viola grown in hydroponics under abiotic stress.

		Mineral nutrients (mg/g dw)								Trace elements (mg/kg dw)		
Cultivar	Treatment (mM NaCl)	C	N	P	S	Na	K	Ca	Mg	Fe	Mn	Zn
Marathon	0	27.19a[A]	40.51a	3.15a	9.26a	3.13b	30.45a	18.08b	4.56a	180.7b	34.5b	9.3a
	80	30.92a	41.08a	3.23a	9.62a	8.34a	27.87b	16.92a	4.53a	193.2a	35.6a	10.5a
	ANOVA *P*-value	n.s.[B]	n.s.	n.s.	n.s.	***	***	n.s.	n.s.	**	*	n.s.
	LSD ($P < 0.05$)	3.78	0.31	0.35	0.20	0.57	0.20	0.60	0.20	3.4	0.7	1.4
Nubia	0	29.01a	40.74a	3.13a	9.32a	2.41b	28.52a	16.58b	4.45a	164.4b	36.5b	10.2a
	80	30.76a	41.09a	3.24a	9.67a	6.59a	25.05b	19.21a	4.44a	211.4a	44.3a	11.6a
	ANOVA *P*-value	n.s.	n.s.	n.s.	n.s.	***	***	*	n.s.	**	*	n.s.
	LSD ($P < 0.05$)	2.00	0.35	0.20	0.35	0.20	0.94	0.75	0.20	3.8	1.3	1.7
Viola	0	24.91a	41.73a	2.49a	9.25b	2.41b	22.69a	14.67a	4.57a	151.9b	23.5b	9.7a
	80	23.92a	41.06a	2.54a	10.43a	10.60a	19.99b	15.04a	4.62a	165.8a	31.0a	10.2a
	ANOVA *P*-value	n.s.	*	n.s.	*	***	*	n.s.	n.s.	*	*	n.s.
	LSD ($P < 0.05$)	3.59	0.23	0.20	0.72	0.20	1.06	0.82	0.20	9.4	1.3	2.1

[A]Means ($n = 3$) within a column (for every cultivar and NaCl treatment) followed by the same lowercase letter are not significantly different at $P < 0.05$ according to Duncan's multiple range test.
[B]Nonsignificant ($P > 0.05$); significant at $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***).

1991; López-Berenguer and others 2006) was present in stalks: Na increased with NaCl as expected, with K decreasing concomitantly. The Ca, Mg, Fe, and Mn concentrations all increased significantly at 80 mM NaCl and remained at similar concentrations in the studied cultivars. Only Zn was not significantly affected by NaCl. The USDA standard reference values for raw broccoli stalks are much lower than those found in this study (Gebhardt and others 2009) for Ca, Fe, Mg, P, K, Na, Zn, and Mn. The levels in the stalks were in a range similar to that in the leaves (Table 9), and only Ca, Fe, and Mn were much higher in the stalks than in the leaves, whereas the levels of Mn and Zn were in the range considered as deficiency, from a physiological point of view (Hanlon and Hochmuth 2000).

The compositions of Nubia and the traditional cultivar Marathon were very similar regarding the mineral and trace elements, while Viola had lower concentrations of certain elements (C, K, and Fe, in leaves and stalks). In general, the variations of minerals and trace elements among cultivars and in a specific organ may be considered minimal. The only remarkable accumulation was found for Ca, Fe, and Mn in the stalks, probably due to the structural importance of this organ in the aerial biomass as well as the potential physiological inactivation involved in the storage of elements at high concentration (Jones and others 1991; Moreno and others 2005).

The influence of the stress imposed by increasing the NaCl concentration in the nutrient solution clearly affected the K/Na balance in the organs studied, as found elsewhere, but the increase or decrease in concentration of any element was not dramatic in any NaCl- or control-treated cultivar. The use of hydroponic growth conditions for broccoli and the application of stress factors (such as NaCl) during plant development may serve to enhance its nutrient load.

Conclusions

The bioactive compounds (glucosinolates, phenolic acids, and flavonoids), nutrients (vitamin C, minerals, and trace elements) and *in vitro* radical-scavenging capacity (DPPH· test) in broccoli varied according to the cultivar (genetic background), organ (physiological role) and salinity stress (80 mM NaCl), for plants grown hydroponically in a greenhouse environment. The abiotic stress (80 mM NaCl) conditioned physiological processes in more-sensitive cultivars (that is, flowering in Viola) and resulted in differences in the phytochemical load of glucosinolates and phenolics (bioactive phytochemicals) and nutrients (vitamin C, N, P, Ca, Mg, Fe, and Mn) in the by-products of the broccoli cultivars Marathon, Nubia, and Viola.

The newly introduced, early, purple sprouting cultivar Viola showed a response that could be related to its physiological difficulty to respond to the imposed stress, and it showed more-unusual results compared with the traditionally grown cultivar Marathon or the well-acclimatized Nubia.

The cultivar Nubia, already grown in the region of study, was not affected dramatically by exposure to 80 mM NaCl and the contents of phytochemicals and nutrients in Nubia broccoli by-products fell within the range of concentrations in edible commercial parts (inflorescences or heads) that have health-promoting effects.

Therefore, the possibility of adding health-promoting value to this inexpensive and otherwise unused agrowaste material is worth the effort of recycling it to obtain bioactive components that could benefit the food and drug industry.

Acknowledgments

The authors thank Dr. David J. Walker, for correction of English in the article. The study was funded by the Regional Agency for Science and Technology of Murcia—Fundación Séneca (Project ref. nr 05588/PI/07). Part of this study was also funded by the Spanish CICYT Natl. Research Programme (AGL2006-6499/AGR). Raúl Dominguez-Perles was funded by a contract from the Spanish Ministry of Science and Innovation (MICINN). We also thank Dr. Fernando Riquelme and Olaya Muñoz (B.Sc.) for their technical support, and the CEBAS-CSIC "La Matanza" Experimental Farm staff for their valuable help and assistance.

References

Agastian P, Kingsley SJ, Vivekanandan M. 2000. Effect of salinity on photosynthesis and biochemical characteristics in mulberry genotypes. Photosynthetica 38:287–90.

Arbos KA, Claro LM, Borges L, Santos CAM, Weffort-Santos AM. 2008. Human erythrocytes as a system for evaluating the antioxidant capacity of vegetable extracts. Nutr Res 28:457–63.

Ben Amor N, Ben Hamed K, Debez A, Grignon C, Abdelly C. 2005. Physiological and antioxidant responses of the perennial halophyte Crithmum maritimum to salinity. Plant Sci 168:889–99.

Boivin D, Lamy S, Lord-Dufour S, Jackson J, Beaulieu E, Coté M, Moghrabi A, Barrette S, Gingras D, Boliveau R. 2009. Antiproliferative and antioxidant activities of common vegetables: a comparative study. Food Chem 112:374–80.

Campas-Baypoli, Sánchez-Machado DI, Bueno-Solano C, Núñez-Gastélum JA, Reyes-Moreno C, López-Cervantes J. 2009. Biochemical composition and physicochemical properties of broccoli flours. Int J Food Sci Nutr 60:163–73.

Campas-Baypoli ON, Sánchez-Machado DI, Bueno-Solano C, Ramírez-Wong B, López-Cervantes J. 2010. HPLC method validation for measurement of sulforaphane level in broccoli by-products. Biomed Chromatogr 24:387–92.

Ciska E, Martyniak-Przybyszewska B, Kozlowska H. 2000. Content of glucosinolates in cruciferous vegetables grown at the same site for two year under different climatic conditions. J Agric Food Chem 48:2862–7.

Davey MW, Van Montagu M, Inzé D, Sanmartin M, Kanellis A, Smirnoff N, Benzie IJJ, Strain JJ, Favell D, Fletcher J. 2000. Plant L-ascorbic acid: chemistry, function, metabolism, bioavailability and effects of processing. J Science Food Agric 80:825–60.

Díaz-Pérez JC. 2009. Root zone temperature, plant growth and yield of broccoli [*Brassica oleracea* (Plenck) var. *italica*] as affected by plastic film mulches. Sci Hortic DOI: 10.1016/j.scienta.2009.08.014.

Eberhardt MV, Kobira K, Keck AS, Juvik JA, Jeffery EH. 2005. Correlation analyses of phytochemical composition, chemical, and cellular measures of antioxidant activity of broccoli (*Brassica oleracea* L. Var. italica). J Agric Food Chem 53:7421–31.

Evers AM. 1994. The influence of fertilization and environment on some nutritionally important quality criteria in vegetables—a review of research in the Nordic countries. Agric Sci Finland 3:177–88.

Farnham MW, Wilson PE, Stephenson KK, Fahey JW. 2004. Genetic and environmental effects on glucosinolate content and chemoprotective potency of broccoli. Plant Breed 123:60–5.

Fenwick GR, Heaney RK. 1983. Glucosinolates and their breakdown products in cruciferous crops, foods and feedingstuffs. Food Chem 11:249–71.

Fink M, Feller C, Scharpf HC, Weier U, Maync A, Ziegler J, Paschold PJ, Strohmeyer K. 1999. Nitrogen, phosphorus, potassium and magnesium contents of field vegetables: recent data for fertiliser recommendation and nutrient balances. J Plant Nutr Soil Sci 162:71–3.

Florez A, Pujolà M, Valero J, Centelles E, Almirall A, Casañas F. 2009. Genetic and environmental effects on chemical composition related to sensory traits in common beans (*Phaseolus vulgaris* L.). Food Chem 113:950–6.

Gebhardt SE, Lemar LE, Pehrsson PR, Exler J, Haytowitz DB, Patterson KK, Nickle MS, Thormas RG, Showell BA, Holden JM. 2009. USDA National Nutrient Database for Standard Reference, Release 22. Available from: http://www.ars.usda.gov/main/site_main.htm?modecode=12-35-45-00. Accessed Mar 24, 2010.

Hanlon FA, Hochmuth GJ. 2000. Reference sufficiency ranges in vegetable crops. Broccoli. In: Sufficiency Ranges for Plant Analysis (SCSB nr 394). Agronomic Division of the N.C. Department of Agriculture and Consumer Services. July 2000. Available from: http://www.agr.state.nc.us/agronomi/saaesd/broccoli.htm. Accessed Mar 24, 2010.

Heimler D, Vignolini P, Dini MG, Vincieri FF, Romani A. 2006. Antiradical activity and polyphenol composition of local Brassicaceae edible varieties. Food Chem 99:464–9.

Hernández Bastida JA, Vela De Oro N, Ortiz Silla R. 2004. Electrolytic conductivity of semiarid soils (southeastern Spain) in relation to ion composition. Arid Land Res Manage 18:265–81.

Howard LA, Wong AD, Perry AK, Klein BP. 1999. β-Carotene and ascorbic acid retention in fresh and processed vegetables. J Food Sci 64:929–36.

Huang C, He W, Guo J, Chang X, Su P, Zhang L. 2005. Increased sensitivity to salt stress in an ascorbate-deficient Arabidopsis mutant. J Exp Bot 56:3041–9.

Jeffery EH, Brown AF, Kurilich AC, Keck AS, Matusheski N, Klein BP, Juvik JA. 2003. Variation in content of bioactive components in broccoli. J Food Composit Anal 16:323–30.

Jones JB Jr, Wolf B, Mills HA. 1991. Plant analysis handbook: a practical sampling, preparation, analysis, and interpretation guide. Athens, GA.: Micro-Macro Publishing. 213 p.

Kurilich AC, Jeffery EH, Juvik JA, Wallig MA, Klein BP. 2002. Antioxidant capacity of different broccoli (Brassica oleracea) genotypes using the oxygen radical absorbance capacity (ORAC) assay. J Agric Food Chem 50:5053–7.

Lester GE. 2006. Environmental regulation of human health nutrients (Ascorbic acid, β-carotene, and folic acid) in fruits and vegetables. Hortscience 41:59–64.

López-Berenguer C, García-Viguera C, Carvajal M. 2006. Are root hydraulic conductivity responses to salinity controlled by aquaporins in broccoli plants? Plant Soil 279:13–23.
López-Berenguer C, Martínez-Ballesta MC, García-Viguera C, Carvajal M. 2008. Leaf water balance mediated by aquaporins under salt stress and associated glucosinolate synthesis in broccoli. Plant Sci 174:321–8.
López-Berenguer C, Martínez-Ballesta MC, Moreno DA, Carvajal M, García-Viguera C. 2009. Growing hardier crops for better health: salinity tolerance and the nutritional value of broccoli. J Agric Food Chem 57:572–8.
Llorach R, Tomás-Barberán FA, Ferreres F. 2004. Lettuce and chicory byproducts as a source of antioxidant phenolic extracts. J Agric Food Chem 52:5109–16.
Mahro B, Timm M. 2007. Potential of biowaste from the food industry as a biomass resource. Engr Life Sci 7:457–68.
Martínez-Ballesta MC, López-Pérez L, Hernández M, López-Berenguer C, Fernández-García N, Carvajal M. 2008. Agricultural practices for enhanced human health. Phytochem Rev 7:251–60.
Martínez-Sánchez A, Allende A, Bennett RN, Ferreres F, Gil MI. 2006. Microbial, nutritional and sensory quality of rocket leaves as affected by different sanitizers. Postharvest Biol Technol 42:86–97.
Moreno DA, Víllora G, Soriano MT, Castilla N, Romero L. 2005. Sulfur, chromium, and selenium accumulated in Chinese cabbage under direct covers. J Environ Manage 74:89–96.
Moreno DA, Carvajal M, López-Berenguer C, García-Viguera C. 2006. Chemical and biological characterisation of nutraceutical compounds of broccoli. J Pharm Biomed Anal 41:1508–22.
Moreno DA, López-Berenguer C, Martínez-Ballesta MC, Carvajal M, García-Viguera C. 2008. Basis for the new challenges of growing broccoli for health in hydroponics. J Sci Food Agric 88:1472–81.
Munns R. 2002. Comparative physiology of salt and water stress. Plant, Cell Environ 25:239–50.
Oh MM, Trick HN, Rajashekar CB. 2009. Secondary metabolism and antioxidants are involved in environmental adaptation and stress tolerance in lettuce. J Plant Physiol 166:180–91.
Podsedek A. 2007. Natural antioxidants and antioxidant capacity of Brassica vegetables: a review. LWT Food Sci Technol 40:1–11.
Premier R. 2002. Phytochemical composition: a paradigm shift for food-health considerations. Asia Pacific J Clin Nutr 11:S197–201.
Proexport. 2008. Valoración de la campaña 2007-2008. In: Proexport: Informe Anual 2007-2008. Pg. 58–62. Available from: www.proexport.es/descargas/GESTION_2007-2008.pdf. Accessed Mar 24, 2010. [In Spanish]
Roy MK, Juneja LR, Isobe S, Tsushida T. 2009. Steam processed broccoli (Brassica oleracea) has higher antioxidant activity in chemical and cellular assay systems. Food Chem 114:263–9.
Scheuner ET, Krumbein A, Schonhof I, Schreiner M. 2005. Increasing the alkyl glucosinolate level in Broccoli by leafstalk infusion of methionine. J Appl Bot Food Qual 79:175–8.
Shannon MC, Grieve CM, Lesch SM, Draper JH. 2000. Analysis of salt tolerance in nine leafy vegetables irrigated with saline drainage water. J Am Soc Hortic Sci 125:658–64.
Vallejo F, Tomás-Barberán FA, García-Viguera C. 2002. Potential bioactive compounds in health promotion from broccoli cultivars grown in Spain. J Sci Food Agric 82:1293–7.
Vallejo F, Tomás-Barberán FA, Gonzalez Benavente-García A, García-Viguera C. 2003. Total and individual glucosinolate contents in inflorescences of eight broccoli cultivars grown under various climatic and fertilisation conditions. J Sci Food Agric 83:307–13.
Wahid A, Ghazanfar A. 2006. Possible involvement of some secondary metabolites in salt tolerance of sugarcane. J Plant Physiol 163:723–30.
West LG, Meyer KA, Balch BA, Rossi FJ, Schultz MR, Haas GW. 2004. Glucoraphanin and 4-Hydroxyglucobrassicin Contents in Seeds of 59 Cultivars of Broccoli, Raab, Kohlrabi, Radish, Cauliflower, Brussels Sprouts, Kale, and Cabbage. J Agric Food Chem 52:916–26.
Wijngaard HH, Roble C, Brunton N. 2009. A survey of Irish fruit and vegetable waste and by-products as a source of polyphenolic antioxidants. Food Chem 116:202–7.
Yan X, Chen S. 2007. Regulation of plant glucosinolate metabolism. Planta 226:1343–52.
Zhou K, Yu L. 2006. Total phenolic contents and antioxidant properties of commonly consumed vegetables grown in Colorado. LWT Food Sci Technol 39:1155–62.