Influence of light on health-promoting phytochemicals of broccoli sprouts

Santiago Pérez-Balibrea, Diego A Moreno and Cristina García-Viguera*

Department of Food Science and Technology, CEBAS-CSIC, Campus Universitario de Espinardo, PO Box 164, Espinardo, E-30100 Murcia, Spain

Abstract

BACKGROUND: Broccoli (Brassicaceae) is a rich source of phytochemicals (glucosinolates and phenolic compounds) and micronutrients (vitamins and minerals). Germinated broccoli sprouts contain much higher levels (10–100 times) of aliphatic (glucoraphanin) and indolic glucosinolates than the inflorescences. This quality characteristic of broccoli sprouts plays an important role in human health and disease prevention. Although it is known that genetic and environmental factors can affect the composition of broccoli inflorescences, the influence of such factors on the seeds and sprouts has not been widely reported. Therefore the aim of this study was to determine the effect of light *versus* dark growth conditions on the phytochemical composition (vitamin C, phenolic compounds and glucosinolates) of broccoli sprouts.

RESULTS: Broccoli sprouts grown in the light were found to have much higher concentrations of vitamin C (by 83%), glucosinolates (by 33%) and phenolic compounds (by 61%) than those grown in the dark. During a 7 day period there was a clear and analogous trend in both treatments, with a general reduction in concentrations over time. Among the different organs studied (seeds, cotyledons, stems and roots), the cotyledons contained the highest levels of bioactive compounds, while the roots contained the lowest.

CONCLUSION: Light treatment of sprouting broccoli seeds increased their concentration of health-promoting phytochemicals, mainly during the first 3–5 days of development. Therefore the younger broccoli sprouts are a better source of bioactive compounds for the consumer than the inflorescences.

Keywords: *Brassica oleracea* var. *italica*; glucosinolates; phenolic compounds; vitamin C; health-promoting compounds

INTRODUCTION

The enrichment of bioactive phytochemicals in plant-based foods suggests the possibility of improving public health through diet. Cruciferous vegetables (*Brassica* spp.), which contain both anticarcinogenic and natural antioxidant bioactive compounds, are excellent examples to illustrate the problem of assessing health benefits of foods.[1]

The content of bioactive compounds in broccoli (*Brassica oleracea* var. *italica*) varies with genotype,[2–4] environmental stress,[5] growth conditions,[6–8] storage and food processing.[1,9–11] For example, glucosinolate levels in brassicas have long been known to vary in response to soil sulfur[7] and nitrogen availability, hydric stress, pests[12] and herbivory.[13,14]

Cruciferous vegetables are an excellent dietary source of glucosinolates and their bioactive degradation products (isothiocyanates), antioxidant vitamins and phenolic compounds.[15,16] Bioactive phytochemicals in brassicas appear to play a determinant role in the prevention of certain diseases and types of cancer.[17,18]

Edible sprouts are one of the potentially new functional foods. Germination is an inexpensive and simple method for improving nutritive value.[19,20] A diet rich in broccoli sprouts provides a good source of indole-3-carbinol, sulforaphane, flavonoids, vitamin C and minerals and may decrease the risk of certain types of cancer.[21,22]

The main objective of this study was to determine the influence of different environmental growth factors (light and dark conditions) on the antioxidant and health-promoting compounds (vitamin C, glucosinolates and phenolic compounds) of broccoli sprouts during the development of the sprouting seeds, until reaching commercially acceptable characteristics (3-, 5- and 7-day-old sprouts).

* Correspondence to: Cristina García-Viguera, Department of Food Science and Technology, CEBAS-CSIC, Campus Universitario de Espinardo, PO Box 164, Espinardo, E-30100 Murcia, Spain
E-mail: cgviguera@cebas.csic.es
Contract/grant sponsor: CICYT National Programme; contract/grant number: AGL2006-6499/AGR
Contract/grant sponsor: CSIC; contract/grant number: 200470E038
Contract/grant sponsor: Juan Marin Enterprise (Campo de Lorca SCL, Murcia, Spain)
Contract/grant sponsor: European Social Fund (ESF)/CSIC
Contract/grant sponsor: European Social Fund (ESF)/Spanish Ministerio de Educación y Ciencia
(*Received 25 April 2007; revised version received 5 October 2007; accepted 19 October 2007*)
Published online 9 January 2008; DOI: 10.1002/jsfa.3169

 J Sci Food Agric 0022–5142/2008/$30.00

MATERIALS AND METHODS

Plant material and experimental conditions

Seeds of broccoli (*B. oleracea* L. [*Italica* group] cv. Marathon) were obtained from Ramiro Arnedo SA (Murcia, Spain), rinsed in distilled water, immersed in 5 g L^{-1} sodium hypochlorite for 2 h and drained. They were then weighed, placed in distilled water and soaked overnight. After pouring off the soaking water, the seeds were weighed and spread evenly on trays (5 g per tray) lined with vermiculite and irrigated with Milli-Q water. Aliquots of 5 g of seeds were frozen in liquid nitrogen and stored at −80 °C pending phytochemical analysis.

The trays were transferred to a controlled environment chamber with a 16 h light/8 h dark cycle and air temperatures of 25 and 20 °C respectively. The relative humidity (RH) was 60% (day) and 80% (night). Photosynthetically active radiation (PAR) of 400 μmol m^{-2} s^{-1} was provided by a combination of fluorescent tubes (Philips TLD 36 W/83, Hamburg, Germany; Sylvania F36 W/GRO, Danvers, Massachusetts, USA) and metal halide lamps (Osram HQI.T 400 W, Munich, Germany).

Broccoli sprouts were produced under two different environmental treatments: artificial light condition ('light', 16 h light/8 h dark photoperiod) and dark condition ('darkness', achieved by completely wrapping the sprouting trays with domestic aluminium foil). For each treatment the sprouts were allowed to grow until they reached 7 days of age. Sprout samples (all sprouts from a single tray, germinated from 5 g of seeds) were collected at different time points (days 3, 5 and 7) after germination. For each day and treatment, three subsamples were rapidly and gently collected, always at 10:00, in the middle of the light period. For each subsample, three replicates were taken for analysis. All subsamples were weighed (fresh mass). The different organs (sprouts, cotyledons, stems or hypocotyls, and roots) were then collected separately, flash frozen in liquid nitrogen and stored at −80 °C pending the analysis of vitamin C, glucosinolates and phenolic compounds.

Extraction and determination of vitamin C

Ascorbic acid (AA) and dehydroascorbic acid (DHAA) contents were determined as described by Vallejo *et al*.[9] Briefly, 200 mg of freeze-dried sample was homogenised in a vortex stirrer for 20 s with 10 mL of extractant solution consisting of methanol/water (5:95 v/v) plus 21 g L^{-1} citric acid, 0.5 g L^{-1} ethylene diamine tetraacetic acid (EDTA) and 0.1 g L^{-1} NaF. The homogenate was filtered through cheesecloth and the pH was adjusted to 2.2–2.4 by addition of 3 mol L^{-1} HCl. The extract was centrifuged (3600 × *g*, 15 min, 4 °C) and the supernatant was recovered, filtered through a Sep-Pak C18 cartridge (Waters, Milford, MA, USA), which had previously been activated with 10 mL of methanol followed by the same volume of water and then the same volume of air, and filtered through a 0.45 μm polyethersulfone filter (Millex-HV13, Millipore, Bedford, MA, USA). High-performance liquid chromatography (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 freshly prepared 1,2-*ortho*-phenylenediamine (OPDA). OPDA solution was added to the water-soluble fraction eluted from a Sep-Pak C18 solid phase extraction cartridge (1:3 v/v). Samples were incubated for 37 min at room temperature in the dark, and 20 μL aliquots were analysed in an HPLC system (Merck-Hitachi, Tokyo, Japan) equipped with an L-4000 UV detector and an L-6000 pump. Separations of DFQ and AA were achieved on a Kromasil 100 C18 column (25 cm × 0.4 cm, 5 μm particle size; Tecnokroma, Barcelona, Spain). The mobile phase was methanol/water (5:95 v/v) containing 5 mmol L^{-1} cetrimide and 50 mmol L^{-1} potassium dihydrogen phosphate at pH 4.5. The flow rate was 0.9 mL min^{-1}. The detector wavelength was initially set at 348 nm; after elution of DFQ the wavelength was manually shifted to 261 nm for AA detection. The DHAA and AA contents were expressed separately as mg DHAA or AA per 100 g fresh weight (FW).

Extraction and determination of phenolic compounds

Freeze-dried powder samples (1 g) were homogenised three times with 25 mL of 700 g L^{-1} methanol. The homogenates were filtered through cheesecloth and kept in ice. The homogenates were subsequently centrifuged (3600 × *g*, 5 min, 4 °C) and the supernatants were evaporated under vacuum at 30 °C to approximately 1 mL, diluted to 2 mL with water and filtered through a 0.45 μm Millex-HV13 filter (Millipore). The extracted samples (20 μL) were analysed in an HPLC system (Waters Cromatografía SA, Barcelona, Spain) consisting of a W600E multisolvent delivery system, an in-line degasser, a W717Plus autosampler and a W2996 photodiode array dectector set at 227 nm, using a Luna C18 column (250 mm × 4.6 mm, 5 μm particle size; Phenomenex, Macclesfield, UK) with a C18-ODS security guard (4 mm × 3 mm) cartridge system (Phenomenex). The mobile phase was a mixture of (A) 1 g L^{-1} Trifluoro acetic acid (TFA) and (B) acetonitrile/TFA (99.9:0.1). Phenolic compounds were eluted off the column in 35 min. The flow rate was 1 mL min^{-1} in a linear gradient starting with 0% B at 0–5 min, reaching 17% B at 15–17 min, 25% B at 22 min, 35% B at 30 min and 50% B at 35 min. Chromatograms were recorded at 280, 320 and 360 nm.

Caffeoyl-quinic acid derivatives were quantified as chlorogenic acid (5-caffeoyl-quinic acid, Sigma, St Louis, MO, USA), flavonoids as quercetin 3-rutinoside (Sigma) and sinapic acid and ferulic derivatives as sinapic acid (Sigma). The total analyte content of phenolic compounds in broccoli sprouts was expressed in mg per 100 g FW.

Extraction and determination of glucosinolates

A modified version of a previously reported procedure,[3] as fully described by Martinez-Sánchez *et al.*,[23] was used for extraction of intact glucosinolates. Briefly, freeze-dried samples (50 mg) were extracted with 1.5 mL of 700 g L^{-1} methanol in a sonicator bath for 10 min, then heated at 70 °C for 30 min in a heating bath, with shaking every 5 min using a vortex stirrer, and centrifuged (17 500 × *g*, 30 min, 4 °C). The supernatants were collected and methanol was completely removed using a rotary evaporator. The dry material obtained was redissolved in 1 mL of ultrapure water and filtered through a 0.45 μm Millex-HV13 filter (Millipore). Each sample (20 μL) was analysed in an HPLC system (Waters Cromatografía SA) under the same conditions as those described above for phenolic compounds. Chromatograms were recorded at 227 nm.

Samples were identified using the previously described intact glucosinolate liquid chromatography/mass spectrometry (LC/MS) method and quantified by HPLC/diode array detection (HPLC/DAD) using sinigrin (sinigrin monohydrate from horseradish, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) as standard.[7] The glucosinolate content was expressed as mg sinigrin equivalent per 100 g FW.

Statistical analysis

All data were subjected to analysis of variance (ANOVA) using the MS-DOS version of Statgraphics 7.0 (Statistical Graphics Corporation and Manugistics, Inc. Rockville (Maryland), USA). The data shown are mean values (mean ± standard deviation). The significance of differences was compared using the least significant difference (LSD) at 99% confidence level.

RESULTS AND DISCUSSION

Our results confirmed the composition of broccoli sprouts according to previously reported data on aliphatic and indolic glucosinolates, phenolic compounds and vitamin C found in broccoli sprouts.[24,25]

Vitamin C

Vitamin C was not detected in unsprouted seeds, in agreement with what was found for other sprouting species such as soybean,[26] while it was present in relatively high amounts in the analysed sprouts. Also, a strong influence of light on the vitamin C content was found (Fig. 1). Thus vitamin C concentrations were higher in the light-grown sprouts than in the dark-grown sprouts (Fig. 1). Among the different organs (cotyledons, stems and roots), the cotyledons showed the highest amount of vitamin C, as expected, with the edible part (cotyledon plus stem) representing

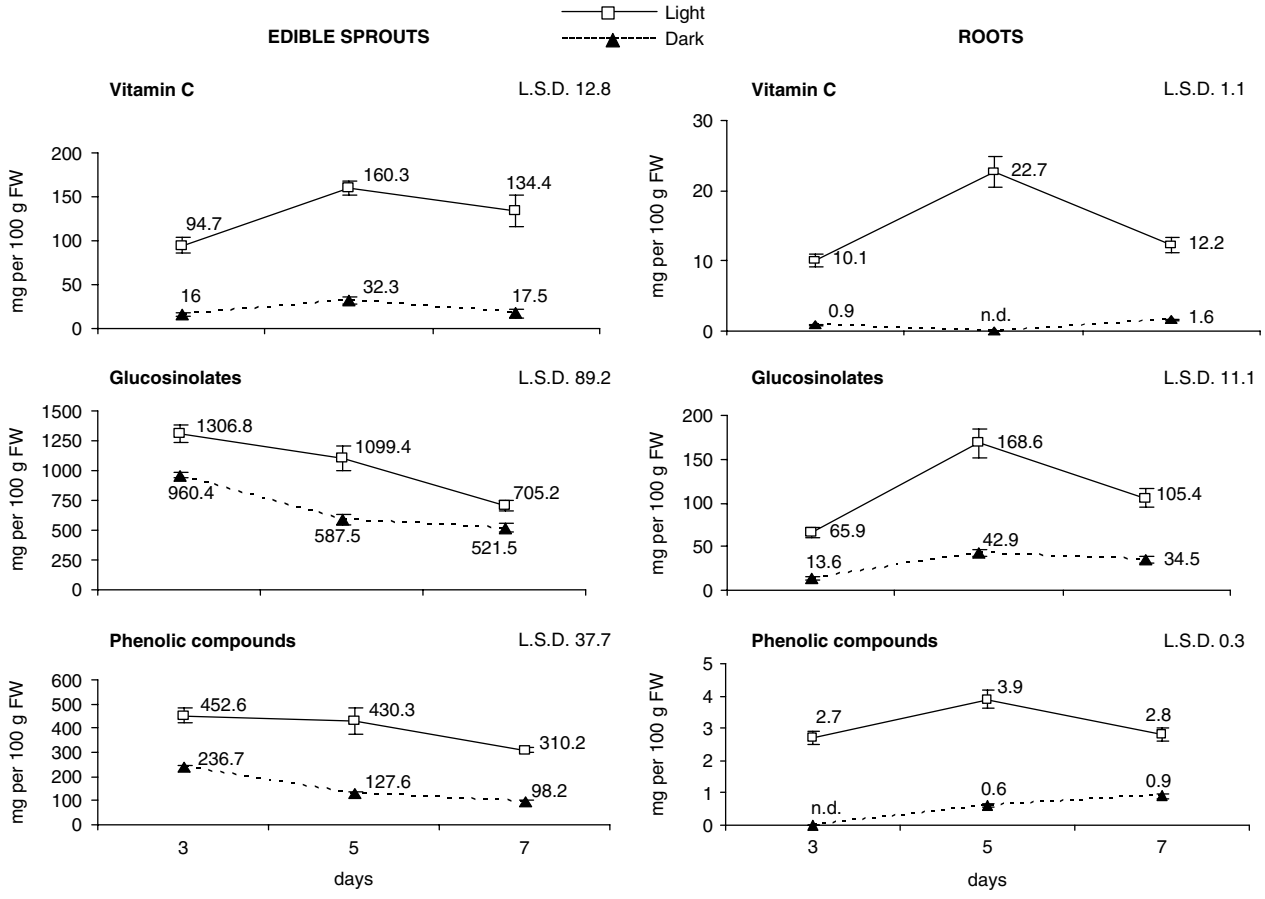

Figure 1. Vitamin C, total glucosinolates and total phenolic compounds in edible broccoli sprouts and roots. Data presented are mean values of three replicates per treatment and time point (mean ± standard deviation). The significance of differences was compared using the least significant difference (LSD) at 99% confidence level.

J Sci Food Agric **88**:904–910 (2008)
DOI: 10.1002/jsfa

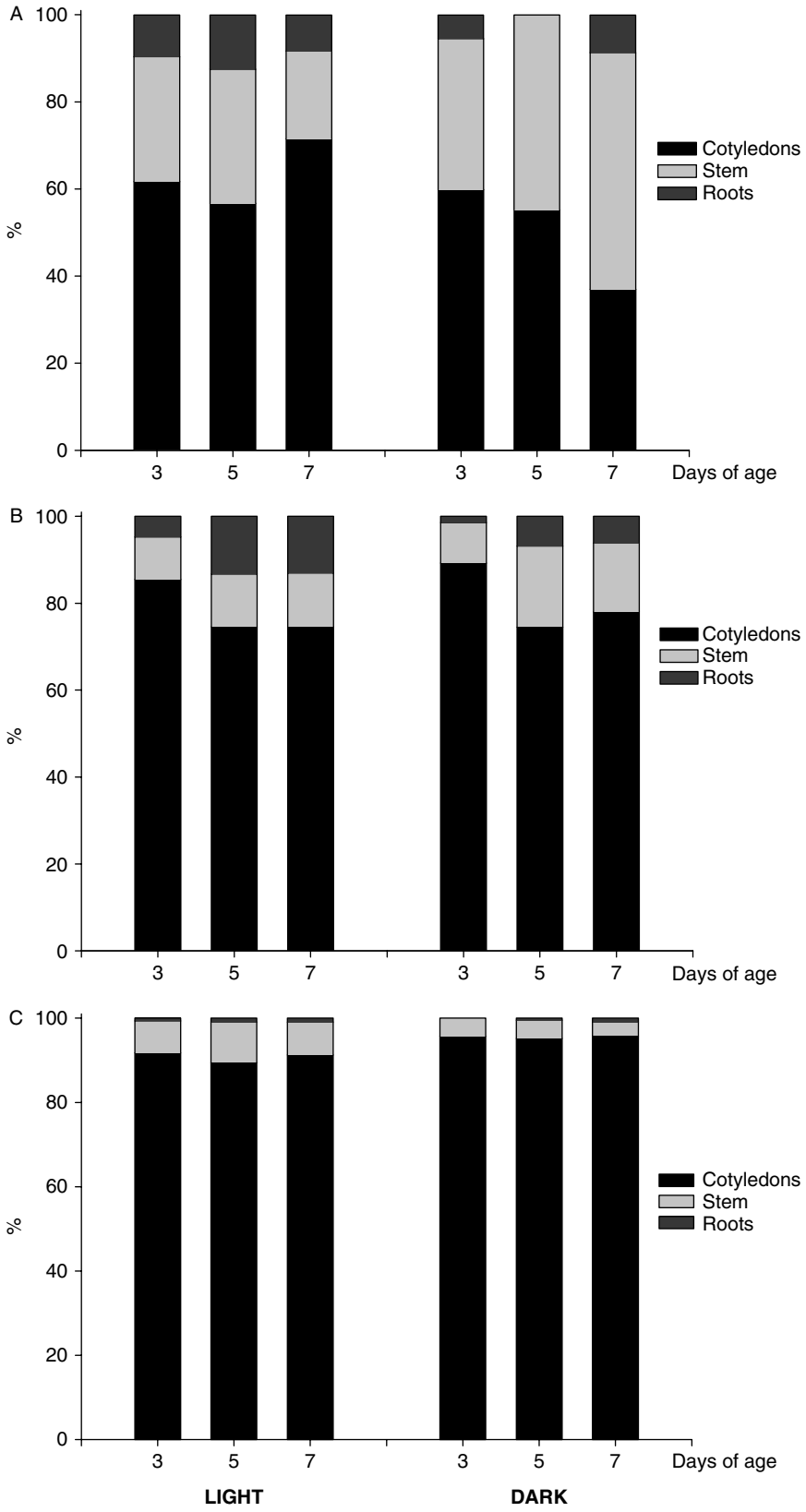

Figure 2. Distribution (%) of total analyte of phytochemicals in different organs of broccoli sprouts: A, vitamin C; B, total glucosinolates; C, total phenolic compounds.

over 93% of the total vitamin C content, while the rest remained in the roots (Fig. 2A). On average, the edible sprouts from seeds germinated under the light contained 83% more vitamin C than the sprouts developed in the dark. The loss in terms of age showed that 3-, 5- and 7-day-old light-grown sprouts had 83, 80 and 87% higher vitamin C contents than the corresponding dark-grown sprouts.

The vitamin C content in the roots mirrored the results for the sprouts, showing higher levels under

light conditions, with a maximum value of 22.7 mg per 100 g FW at 5 days of age (Fig. 1).

The vitamin C content in the light-grown edible sprouts was higher than that previously reported in the inflorescences.[7,9,27] In commercial broccoli inflorescences the vitamin C concentration ranged from 52 mg per 100 g FW[27] to 103 mg per 100 g FW,[7] while the vitamin C concentration in 'Marathon' inflorescences was 75 mg per 100 g FW.[9] The vitamin C content in the sprouts (Fig. 1) is favoured by the development of the sprouting seed, which involves the reactivation of vitamin C biosynthesis,[26] and the possible implications of this bioactive compound in the synthesis of secondary metabolites and photoprotection[28] could explain the higher vitamin C levels under light conditions.[29] Therefore the control of the light regime offers possibilities for improving the nutritional quality of broccoli sprouts by increasing their vitamin C content. Broccoli sprouts grown under light conditions seem to be a good source of vitamin C, with higher concentrations than the edible portion of the broccoli heads.

Glucosinolates

The total glucosinolate concentration was higher in ungerminated seeds than in sprouts (Fig. 1). On the other hand, it was significantly lower (33% on average) in the dark-grown sprouts than in the light-grown sprouts, and the variation between samplings followed the trend 3-day-old > 5-day-old > 7-day-old sprouts. In the light-grown sprouts the glucosinolate content decreased by 16% from day 3 to day 5 and showed a total loss of 46% from day 3 to day 7 (Fig. 1). In addition, the edible part presented the highest amount of these compounds, 93% of the total detected (Fig. 2B), owing to the high concentrations found in the cotyledons (1172, 945 and 605 mg per 100 g FW for 3-, 5- and 7-day-old sprouts respectively). The glucosinolate levels in the dark-grown sprouts were significantly lower. Thus glucosinolates were affected by the light/dark conditions and the developmental stage of the sprouts, with glucosinolate contents decreasing over time. According to previous research, no large changes in glucosinolate content throughout the day could be expected if the environmental parameters (air temperature, relative humidity and light) are kept constant at an optimal growth and development temperature.[8]

The data presented in Fig. 1 also show that the roots from sprouts cultivated under light conditions had higher glucosinolate contents than the roots from sprouts grown under dark conditions. The maximum glucosinolate concentration in the roots was recorded at 5 days of age under both conditions. The downward trend of the glucosinolate concentration in the aerial part is opposite to what happened in the roots. The explanation for this may be a translocation from shoots to roots to support growth under the experimental conditions tested, or possibly a dilution effect in the aerial part. Further research is needed to fully understand these phenomena.

Edible sprouts showed glucosinolate contents ranging from 1306 to 705 mg per 100 g FW under light conditions and from 960 to 521 mg per 100 g FW under dark conditions. These amounts are considerably larger than those found in the inflorescences at different developmental stages[6,7,9] and also higher than in 11-day-old broccoli sprouts.[25] This is due to the high glucosinolate contents in the earlier developmental stages and the decrease with aging of the sprouts[30] as confirmed by previous studies, where non-germinated seeds had the highest glucosinolate levels,[31] which could lead to greater induction of Phase II detoxication enzymes.[8]

Phenolic compounds

Our results confirmed the phenolic profile of broccoli in terms of caffeoyl-quinic acid and sinapic acid derivatives as determined by HPLC/DAD/MS (data not shown).[7,9] The variation in content of total phenolic compounds in the edible part and roots of broccoli sprouts is shown in Fig. 1. As found when studying glucosinolates, the seeds presented the highest total concentration of these health-promoting compounds (546.5 mg per 100 g FW), and 17 and 56% losses occurred under light and dark sprouting conditions respectively. Again, the cotyledons were the richest organs in both treatments and at different samplings, with the edible part representing 99% of the total concentration of these compounds (Fig. 2C). The tendency over the 7 day growth period was analogous in both light and dark treatments, with phenolic contents decreasing over time (Fig. 1). Also, a higher content of phenolics was detected in sprouts germinated under light conditions. A similar behaviour was observed in sprouting seeds of legumes.[32] The light received during sprouting could trigger the phenolic content by promoting photosynthesis and the malonyl-CoA pathway, which is related to the synthesis of phenolic compounds in sprouts.[32] On the other hand, over the 7 day period of study the roots showed the opposite trend to the aerial part, suggesting a translocation and redistribution of phenolic compounds within the seedlings over time.

The phenolic content in the broccoli sprouts was higher than that previously reported reported in the inflorescences.[9,27] Edible broccoli sprouts presented total phenolic contents ranging from 453 to 310 mg per 100 g FW under light conditions and from 237 to 98 mg per 100 g FW under dark conditions. These amounts are 10–40 times higher than those found in the inflorescences of different cultivars of broccoli.[9] Moreover, at different developmental phases throughout the productive period of broccoli, we also found higher concentrations of phenolic compounds in the sprouts compared with those reported previously.[7] Thus broccoli sprouts are a good source of phenolics, being richer than broccoli inflorescences in these health-promoting compounds.

J Sci Food Agric **88**:904–910 (2008)
DOI: 10.1002/jsfa

CONCLUSIONS

We confirmed that there was a clear benefit of light conditions over dark conditions on the phytochemical composition of broccoli sprouts in terms of vitamin C, glucosinolates and phenolic compounds. Light favoured the concentration of bioactive phytochemicals in the sprouting seeds, mainly during the first 3–5 days of development. Thus the younger sprouts are a better source of chemoprotective compounds for the consumer than the inflorescences, supplying 7% more glucosinolates, 68% more vitamin C and 25% more phenolic compounds per 100 g serving.

ACKNOWLEDGEMENTS

The authors wish to thank the CICYT National Programme for financial support of this work (AGL2006-6499/AGR). Part of the work was also funded by the CSIC (Proyecto Intramural 200470E038) and the Juan Marin Enterprise (Campo de Lorca SCL, Murcia, Spain). Santiago Pérez-Balibrea thanks the European Social Fund (ESF) and the CSIC for the I3P postgraduate contract. Diego A Moreno thanks the European Social Fund (ESF) and the Spanish Ministerio de Educación y Ciencia for funding through the 'Ramon y Cajal' S&T Programme.

REFERENCES

1 Jeffery EH, Brown AF, Kurilich AC, Keck AS, Matusheski N, Klein BP, *et al*, Variation in content of bioactive components in broccoli. *J Food Compos Anal* **16**:323–330 (2003).
2 West LG, Meyer KA, Balch BA, Rossi FJ, Schultz MR and Haas GW, 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**:916–926 (2004).
3 Bennet RN, Mellon FA and Kroon PA, Screening crucifers seeds as sources of specific intact glucosinolates using ion-pair high-performance liquid chromatography negative ion electrospray mass spectrometry. *J Agric Food Chem* **52**:428–438 (2004).
4 Charron CS, Saxton AM and Sams CE, Relationship of climate and genotype to seasonal variation in the glucosinolate–myrosinase system. II. Myrosinase activity in ten cultivars of *Brassica oleracea* grown in fall and spring seasons. *J Sci Food Agric* **85**:682–690 (2005).
5 López-Berenguer C, García-Viguera C and Carvajal M, Are root conductivity responses to salinity controlled by aquaporins in broccoli plants? *Plant Soil* **279**:13–23 (2006).
6 Rangkadilok N, Nicolas ME, Bennett RN, Eagling DR, Premier RR and Taylor PWJ, The effect of sulfur fertilizer on glucoraphanin levels in broccoli (*B. oleracea* L. var. *italica*) at different growth stages. *J Agric Food Chem* **52**:2632–2639 (2004).
7 Vallejo F, García-Viguera C and Tomás-Barberán FA, Changes in broccoli (*Brassica oleracea* var. *italica*) health-promoting compounds with inflorescence development. *J Agric Food Chem* **51**:3776–3782 (2003).
8 Rosa EAS and Rodrigues PMF, The effect of light and temperature on glucosinolates concentration in the leaves and roots of cabbage seedlings. *J Sci Food Agric* **78**:208–212 (1998).
9 Vallejo F, Tomás-Barberán FA and García-Viguera C, Glucosinolates and vitamin C content in edible parts of broccoli inflorescences after domestic cooking. *Eur Food Res Technol* **215**:310–316 (2002).
10 Dekker M and Verkerk R, Dealing with variability in food production chains: a tool to enhance the sensitivity of epidemiological studies on phytochemicals. *Eur J Nutr* **42**:67–72 (2003).
11 Jones RB, Faragher JD and Winkler S, A review of the influence of postharvest treatments on quality and glucosinolate content in broccoli (*Brassica oleracea* var. *italica*) heads. *Postharv Biol Technol* **41**:1–8 (2006).
12 Bennet R and Wallsgrove R, Secondary metabolites in plant defence mechanisms. *New Phytol* **127**:617–633 (1994).
13 Zhao F, Evans EJ, Bilsborrow PE and Syers JK, Influence of nitrogen and sulphur on the glucosinolate profile of rapeseed (*Brassica napus* L). *J Sci Food Agric* **64**:295–304 (1994).
14 Champoliver L and Merrien A, Effects of water stress applied at different growth stages to *Brassica napus* L. var. *oleifera* on yield components and seed quality. *Eur J Agron* **5**:153–160 (1996).
15 Jeffery EH and Jarrel V, Cruciferous vegetables and cancer prevention, in *Handbook of Nutraceuticals and Functional Foods*, ed. by Wildman REC. CRC Press, Boca Raton, FL, pp. 169–191 (2001).
16 Jagdish S, Rai M, Upadhyay AK, Bahadur A, Chaurasia SNS and Sing KP, Antioxidant phytochemicals in broccoli (*Brassica oleracea* var. *italica* Plenck) cultivars. *J Food Sci Technol* **43**:391–393 (2006).
17 Kris-Etherton PM, Hecker KD, Bonanome A, Coval SM, Binkoskin AE, Hilpert KF, *et al*, Bioactive compunds in foods: their role in the prevention of cardiovascular disease and cancer. *Am J Med* **113**:71S–88S (2002).
18 Krinsky NI, Beecher GR, Burk RF, Chan AC, Erdman JW, Jacob RA, *et al*, *Dietary References Intakes for Vitamin C, Vitamin E, Selenium and Carotenoids*. National Academy Press, Washington, DC (2000).
19 Honke J, Kozłowska H, Vidal-Valverde C, Frias J and Górecki R, Changes in quantities of inositol phosphates during maturation and germination of legume seeds. *Z Lebensm Untersuch Forsch A* **206**:279–283 (1998).
20 Zhang H, Vasanthan T and Wettasinghe M, Enrichment of tocopherols and phytosterols in canola oil during seed germination. *J Agric Food Chem* **55**:355–359 (2007).
21 Lindsay DG and Astley SB, European research on the fundamental effects of dietary antioxidants. *Mol Aspects Med* **23**:1–38 (2002).
22 Fahey JW, Zhang Y and Talalay P, Broccoli sprouts: an exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. *Proc Natl Acad Sci USA* **94**:10367–10372 (1997).
23 Martinez-Sánchez A, Allende A, Bennett RN, Ferreres F and Gil MI, Microbial, nutritional and sensory quality of rocket leaves as affected by different sanitizers. *Postharv Biol Technol* **42**:86–97 (2006).
24 Moreno DA, Carvajal M, López-Berenguer C and García-Viguera C, Chemical and biological characterisation of nutraceutical compounds of broccoli. *J Pharmaceut Biomed* **41**:1508–1522 (2006).
25 Aires E, Rosa E and Carvalho R, Effect of nitrogen and sulfur fertilization on glucosinolates in the leaves and roots of broccoli sprouts (*Brassica oleracea* var. *italica*). *J Sci Food Agric* **86**:1512–1516 (2006).
26 Xu MJ, Dong JF and Zhu MY, Effects of germination conditions on ascorbic acid level and yield of soybean sprouts. *J Sci Food Agric* **85**:943–947 (2005).
27 Singh J, Upadhyay AK, Prasad K, Bahadur A and Rai M, Variability of carotenes, vitamin C, E and phenolics in *Brassica* vegetables. *J Food Compos Anal* **20**:106–112 (2007).
28 Wolucka BA, Goossens A and Inzé D, Methyl jasmonate stimulates the *de novo* biosynthesis of vitamin C in plant cell suspensions. *J Exp Bot* **56**:2527–2538 (2005).

29 Franceschi VR and Tarlyn NM, L-Ascorbic acid is accumulated in source leaf phloem and transported to sink tissues in plants. *Plant Physiol* **130**:649–656 (2002).
30 Pereira FMV, Rosa E, Fahey JW, Stephenson KK, Carvalho R and Aires A, Influence of temperature and ontogeny on the levels of glucosinolates in broccoli (*Brassica oleracea* var. *italica*) sprouts and their effect on the induction of mammalian phase 2 enzymes. *J Agric Food Chem* **50**:6239–6244 (2002).
31 Rosa EAS, Heaney RK, Portas CAM and Fenwick GR, Changes in glucosinolate concentrations in *Brassica* crops (*Brassica oleracea* and *Brassica napus*) through growth seasons. *J Sci Food Agric* **71**:237–244 (1996).
32 Kim EH, Kim SH, Chung JI and Chi HY, Analysis of phenolic compounds and isoflavones in soybean seeds (*Glycine max* (L.) Merill) and sprouts grown under different conditions. *Eur Food Res Technol* **222**:201–208 (2006).

J Sci Food Agric **88**:904–910 (2008)
DOI: 10.1002/jsfa