Food Chemistry 127 (2011) 192–196

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Short communication

# No *de novo* sulforaphane biosynthesis in broccoli seedlings

Antonie Gorissen [a,*], Nicolai U. Kraut [b], Ries de Visser [a], Marcel de Vries [b], Han Roelofsen [b], Roel J. Vonk [b]

[a] IsoLife BV, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands
[b] Centre for Medical Biomics, University Medical Centre Groningen, Groningen, The Netherlands, Hanzeplein 1, 9713 GZ Groningen, The Netherlands

ARTICLE INFO

*Article history:*
Received 14 November 2010
Accepted 10 December 2010
Available online 17 December 2010

*Keywords:*
Broccoli
Seedlings
Stable isotope labelling
$^{13}C$, Sulforaphane
Glucosinolates

ABSTRACT

The isothiocyanate sulforaphane, present in significant amounts in broccoli (*Brassica oleracea* L.) seedlings in the form of its precursor glucoraphanin, has been identified as an inducer of quinine reductase, a phase-II detoxification enzyme known for its anticarcinogenic properties. Its concentration in broccoli seedlings usually decreases during the first 7–14 days after germination. No conclusive data on sulforaphane metabolism in seedlings are available in the literature. Here, we unambiguously demonstrate in $^{12}C/^{13}C$-cross experiments that sulforaphane is not biosynthesised *de novo* during the first week of seedling development. Both $^{12}C$ (99 atom% $^{12}C$) and $^{13}C$ (98 atom% $^{13}C$) broccoli seeds were produced and subsequently germinated and grown either in a $^{13}CO_2$ or a $^{12}CO_2$ environment. Afterwards, the labelling degree of sulforaphane in seeds and in seedlings was analysed by HPLC–MS. We conclude that sulforaphane exclusively originates from seed reserves and that *de novo* biosynthesis is not detectable (<1%) in broccoli seedlings.



## 1. Introduction

Glucosinolates are secondary plant metabolites in *Brassica* vegetables exhibiting various important ecological and nutritional properties. During the last decades of the 20th century, deleterious anti-nutritional properties in animal feed containing glucosinolates have been recognised, resulting in breeding programs substantially reducing glucosinolate concentrations in, for example, *Brassica napus* (Fenwick, Heany, & Mullin, 1983). Positive effects of glucosinolates and their degradation products such as (iso)thiocyanates and nitriles have also been reported, e.g., an improved resistance to fungal infestation and insect pests (Koritsas, Lewis, & Fenwick, 1991; Mithen, 1992) and concurrently, consumption of green and yellow vegetables were found to be associated with lower death rates caused by cancer (Colditz et al., 1985). Glucosinolates were gradually discovered to be phytochemicals exhibiting anti-cancer properties; Zhang, Talalay, Cho, and Possner (1992) identified sulforaphane ($C_6H_{11}NOS_2$), originating from glucoraphanin in broccoli, as a strong inducer of quinine reductase, a phase II detoxification enzyme. In a recent review, it was suggested that about 3–5 servings of broccoli per week seems to be cancer preventive (Herra & Büchler, 2010).

Nowadays, many commercial broccoli products are available on the market, one of them being broccoli seedlings, often less than 1 week old. Several studies reported a decrease in glucoraphanin/sulforaphane concentration during the growth of seedlings from 2 to 14 days (Lim, Lee, & Kim, 2009; Nakagawa et al., 2006; Pérez-Balibrea, Moreno, & García-Viguera, 2008; Rychlik & Adam, 2008; Sivakumar, Aliboni, & Bacchetta, 2007). Although Sivakumar et al. (2007) mentioned that no explanation was known for the higher accumulation of sulforaphane in young seedlings and the decrease during growth, others stated that this possibly resulted from a dilution effect caused by growth of the cotyledons (Lim et al., 2009; Pérez-Balibrea et al., 2008). No explanation was given by Pérez-Balibrea et al. (2008) for the higher concentration in broccoli seedlings grown in the light compared with seedling (sprouts) grown in the dark, but the difference suggests that under light conditions new sulforaphane is biosynthesised during germination and growth of the broccoli seedlings.

The aim of this study was to investigate, using a unique stable isotope technique, if sulforaphane is newly biosynthesised after germination during broccoli seedling growth. By uniform (>98 atom% $^{13}C$) labelling of broccoli plants to yield $^{13}C$ seeds, sulforaphane-C originating from other sources (atmosphere) could be conclusively shown to be absent in growing seedlings. Here, we present evidence that sulforaphane is not biosynthesised *de novo* in broccoli seedlings but originates solely from seed reserves.

## 2. Materials and methods

### 2.1. Materials

#### 2.1.1. Plants and labelling facility ESPAS

Broccoli (*Brassica oleracea* L.) seeds, from which the commercial product Broccocress® is grown, were germinated and plants were

* Corresponding author. Tel.: +31 317 480508; fax: +31 317 418094.
*E-mail address:* ton.gorissen@isolife.nl (A. Gorissen).

0308-8146/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2010.12.067

hydroponically grown in the ESPAS (Experimental Soil Plant Atmosphere System) facility (Gorissen, Kuikman, Van Ginkel, Van de Beek, & Jansen, 1996), using unique hermetically-sealed plant growth chambers with a volume of 3500 L, specifically re-designed for atmospheric high abundance $^{13}C$-isotope labelling (IsoLife BV, Wageningen, The Netherlands). Two experiments were performed, one in which normal '$^{12}C$'-seeds were germinated and cultivated in a $^{13}C$–$CO_2$ atmosphere and a second, reversed experiment in which uniformly (>98 atom%) $^{13}C$-labelled broccoli seeds were germinated and cultivated in a $^{12}C$–$CO_2$ atmosphere.

*2.1.2. Reagents and apparatus*

Methanol Chromasolv® for LC–MS and sulforaphane (standard) were purchased from Sigma–Aldrich, Steinheim am Albuch, Germany. Formic acid was supplied by Fluka, Steinheim am Albuch, Germany. Ultrapure water was prepared on the day of analysis using a Sartorius arium® 611 (Sartorius AG, Göttingen, Germany).

A Shimadzu UFLC® (Shimadzu, Kyoto, Japan) was coupled *via* a Shimpack XR-ODS column (2.2 μm, 75 × 3 mm) (Shimadzu) with an H-ESI mounted on a Thermo Electron LTQ Orbitrap XL® (ThermoFisher Scientific, Bremen, Germany).

*2.2. Methods*

*2.2.1. Plant growth*

*2.2.1.1. Experiment 1: Broccoli seedling growth from $^{12}C$-seeds in a $^{13}C$–$CO_2$ atmosphere.* Regular ('$^{12}C$') broccoli seeds (two breeding lines, BC1 and BC2 provided by Koppert Cress BV, The Netherlands) were germinated and subsequently cultivated in 3-L pots containing inert fine gravel in a uniformly $^{13}C$–$CO_2$ labelled atmosphere (98 atom% $^{13}C$, 2 atom% $^{12}C$ instead of the natural atmospheric $^{13}C$–$CO_2$ abundance of 1.1 atom% $^{13}C$ and 98.9 atom% $^{12}C$), using $^{13}CO_2$ from pressurised cylinders (Isotec, Inc., Miamisburg, OH). The environmental and atmospheric conditions in the ESPAS facility were fully controlled. Seedlings were grown at a light intensity of *ca.* 600 μmol $m^{-2}$ $s^{-1}$ during 16-h day, a day:night temperature of 22:15 °C and RH of 75%. The plants were cultivated on a 0.2 strength Steiner nutrient solution (Steiner, 1984). After 11 days, some broccoli seedlings were harvested and subsequently analysed for both $^{12}C$- and $^{13}C$-sulforaphane and its precursor glucoraphanin (see Section 2.2.2). Some broccoli seedlings of the BC2-line were further uniformly $^{13}C$-labelled until plant maturity. After about 8 months growth in the ESPAS facility, fully ripened seeds were harvested and kept at 4 °C to vernalise for at least 2 months prior to their use in Experiment 2.

*2.2.1.2. Experiment 2: Broccoli seedling growth from $^{13}C$-seeds in a $^{12}C$–$CO_2$ atmosphere.* Uniformly $^{13}C$-labelled broccoli seeds (98.2 atom% $^{13}C$, see Section 3) and non-labelled $^{12}C$-broccoli seeds (both the BC2-line) were germinated and subsequently cultivated for 7 days in a normal $^{12}C$–$CO_2$ atmosphere containing natural background concentrations of $^{13}CO_2$ (1.1 atom%), after which they were also analysed for both $^{12}C$- and $^{13}C$-sulforaphane and its precursor glucoraphanin.

*2.2.2. Sample preparation*

*2.2.2.1. Broccoli seed.* Both $^{12}C$- and $^{13}C$-seeds (~4 mg) were homogenised in 600 μL ultrapure water with a mortar and pestle, 25-fold diluted with 0.1% formic acid and subsequently 2 μL was injected into the LC–ESI-HRMS.

*2.2.2.2. Broccoli seedlings.* Fresh seedlings (~30 mg) were homogenised in 500 μL of ultrapure water with a mortar and pestle, incubated for 30 min at 37 °C to activate the plant's own myrosinase, a thioglucoside glucohydrolase, acidified by adding 50 μL 1 M HCl and further incubated for 4 h at 42 °C. The suspension was centrifuged at 2000*g* for 5 min; 10 μL of the supernatant were diluted to 100 and 1 μL was injected into the LC–ESI-HRMS.

*2.2.3. HPLC–MS analysis*

*2.2.3.1. Liquid chromatography.* The following solvent system was used for separation of the sulforaphane and other metabolites. Solvent **A** was water (obtained via arium® 611UFD Ultrapure Water System, Sartorius, Germany) and solvent **B** was acetonitrile (Biosolve, Valkenswaard, The Netherlands), both containing 0.1% v/v formic acid (Fluka, Sigma–Aldrich, St. Louis, MO); the flow rate was 0.5 mL/min. The gradient was as follows: 5% **B** at 0 min to 80% **B** at 7 min.

*2.2.3.2. Mass spectrometry.* Sample analysis was carried out in positive ion detection mode. The capillary temperature and the heated interface were at 300 °C and the sheath and auxiliary gas flow rates were 50 and 40 (arbitrary units). The mass range was set to 150–600 Da at a resolution of 30,000 at *m/z* 400. Thermo Xcalibur 2.0 software was used for the qualitative analysis of the generated data.

*2.2.3.3. Calculation of theoretical molecular mass distribution from isotopomer abundance.* The main isotopes affecting molecular mass of organic compounds are $^{12}C/^{13}C$ (current label), $^{14}N/^{15}N$, $^{16}O/^{18}O$ (3% in $^{13}CO_2$) and $^{32}S/^{33}S/^{34}S$. Theoretically expected mass distributions were calculated by summing the relative abundances of the various isotopomers for each molecular mass (*m/z*), as derived from known isotope abundances by using the binomial distribution (Jennings & Matthews, 2005).

## 3. Results and discussion

*3.1. Results*

Broccoli seedling biomass (g dry wt/seedling) at 7–11 days after germination was 2 to 3-fold the initial seed dry weight (results not shown), leading to an expected carbon isotope dilution from 98% down to 30–50%. Fig. 1 shows the mass distribution of non-labelled $^{12}C$-sulforaphane ($C_6H_{11}NOS_2$) – originating from $^{12}C$-seeds grown in a 98 atom% $^{13}C$–$CO_2$ atmosphere – with the [M+H$^+$] signal at *m/z* 178.04. The dominant signal at the expected *m/z* value of 178.04 for $^{12}C_6$-sulforaphane, and the minor signals at [M+H$^+$] + 1 to [M+H$^+$] + 6, show that the broccoli was not enriched with $^{13}C$ (as compared to a $^{12}C$ standard). The mass spectrum in Fig. 2 shows the [M+H$^+$] peaks of $^{13}C_6$-sulforaphane from $^{13}C$-seedlings at 178.04 + 6 = *m/z* 184.05 and $^{13}C_5{}^{12}C$-sulforaphane at *m/z* 183.05 in a ratio of 89:11. This ratio indicates an overall $^{13}C$-abundance of sulforaphane-C in the labelled seeds of 98.2 atom%, calculated using the binomial distribution (Hellerstein & Neese, 1999; Jennings & Matthews, 2005). Consistent with this result, virtually no $^{12}C$-sulforaphane (178.04 *m/z*) was detected in $^{13}C$-seedlings and no $^{13}C$-sulforaphane in $^{12}C$-seedlings. The other small signal with an *m/z* value of 186.05 mainly refers to the isotopomer containing six $^{13}C$ carbon atoms, and additionally one $^{18}O$ instead of a $^{16}O$ or one $^{34}S$ atom instead of $^{32}S$. For an overview of experimental and theoretically expected abundances, see Table 1.

The mass distribution of $^{13}C_9$-phenylalanine in broccoli seedlings originating from $^{13}C$-seeds grown in a 98 atom% $^{12}C$–$CO_2$ atmosphere is shown in Fig. 3 and of $^{12}C_9$-phenylalanine in broccoli seedlings originating from $^{12}C$-seeds grown in a 98 atom% $^{13}C$–$CO_2$ atmosphere in Fig. 4. The calculations presented in Table 2 show that in Experiment 1 the enrichment of phenylalanine in the soluble fraction had been increased to 94.7 atom% $^{13}C$ and in Experiment 2 decreased to 3.2 atom% $^{13}C$. This clearly shows that the

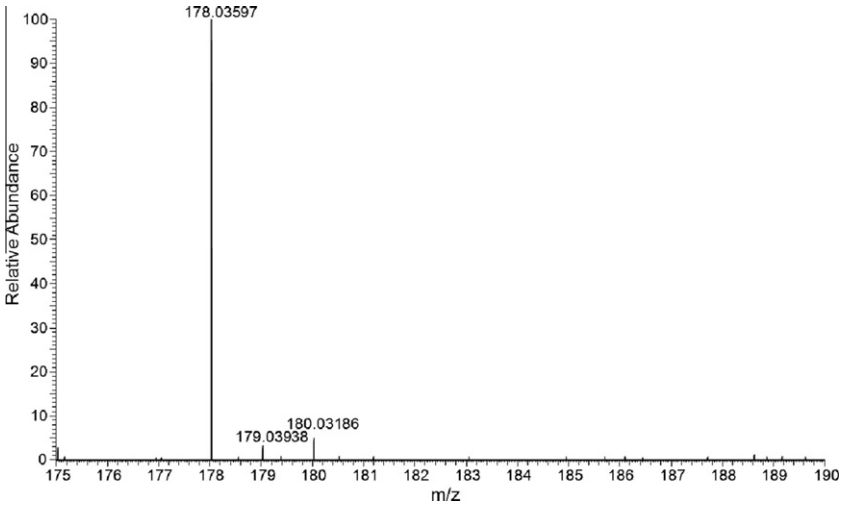


**Fig. 1.** High resolution mass spectrum (*m/z* 175.0–190.0, [$^{12}C_6H_{12}ONS_2 + H^+$] mass 178.04) of sulforaphane in broccoli seedlings grown from $^{12}C$-seeds in a $^{13}C$–$CO_2$ atmosphere.

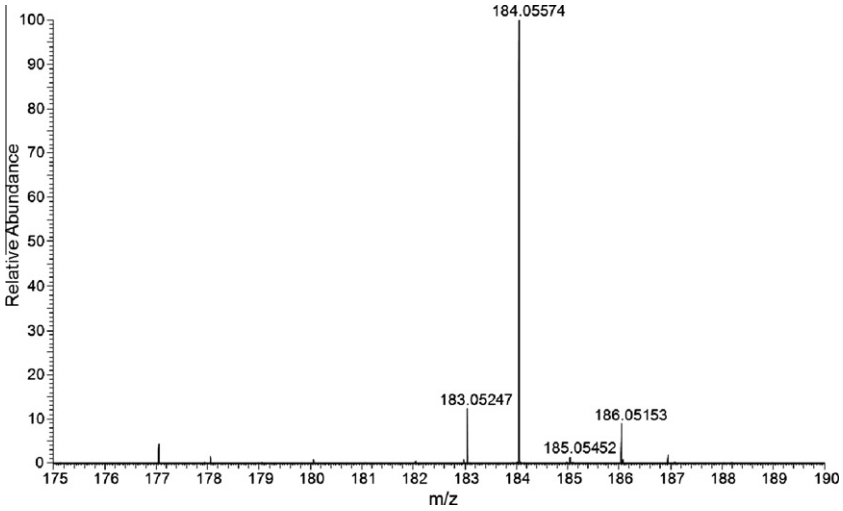


**Fig. 2.** High resolution mass spectrum (*m/z* 175.0–190.0, [$^{13}C_5{}^{12}CH_{12}ONS_2 + H^+$] mass 183.05 and [$^{13}C_6H_{12}ONS_2 + H^+$] mass 184.05) of sulforaphane in broccoli seedlings grown from $^{13}C$-seeds in a $^{12}C$–$CO_2$ atmosphere.

**Table 1**
Mass distributions of $^{12}C$- and $^{13}C$-sulforaphane mass isotopomers in Experiments 1 and 2. Theoretically expected masses are shown for the case of absence of turnover; experimental data are derived from Figs. 1 and 2.

| | *m/z* of Sulforaphane mass isotopomers | | | |
|---|---|---|---|---|
| | M − 1 | M = M + H$^+$ | M + 1 | M + 2 |
| *Experiment 1* | | | | |
| $^{12}C$ Theoretically expected | – | 100 | 8.7 | 9.3 |
| $^{12}C$ Experimental result | – | 100 | 4.0 | 6.0 |
| *Experiment 2* | | | | |
| $^{13}C$ Theoretically expected | 14.0 | 100 | – | 9.0 |
| $^{13}C$ Experimental result | 12.2 | 100 | – | 11.9 |

amino acid phenylalanine, in contrast to sulforaphane, had been synthesised almost completely *de novo* at harvest time.

### 3.2. Discussion

Although the variation in overall glucosinolate content in leaves of *Brassica* sp. is considerable during the growing season (Li, Kiddle, Bennett, Doughty, & Wallsgrove, 1999; Porter, Morton, Kiddle, Doughty, & Wallsgrove, 1991), the dynamics of the sulforaphane concentration in broccoli seedlings usually show a predictable pattern. During this stage, the sulforaphane concentrations in the cotyledons tend to decrease during the first 14 days (Lim et al., 2009; Sivakumar et al., 2007). Nakagawa et al. (2006), Sivakumar et al. (2007), and Pérez-Balibrea et al. (2008) all mentioned that little is known about the relationship between seedling growth and sulforaphane concentration. However, Bennett, Ludwig-Muller, Kiddle, Hilgenberg, and Wallsgrove (1995) had already shown that mono-oxygenases catalysing glucosinolate biosynthesis were lacking in the cotyledons of both Chinese cabbage and oilseed rape, indirectly demonstrating that glucosinolate biosynthesis and thus sulforaphane production in the cotyledon leaves cannot occur using this pathway.

In our first experiment ($^{12}C$-seed in a $^{13}C$–$CO_2$ atmosphere), only $^{12}C$-sulforaphane was detected in the developing seedlings, whereas the only available $^{12}C$ source in the system was formed by the broccoli seeds. In the second, reversed, experiment ($^{13}C$-seed in a $^{12}C$–$CO_2$ atmosphere) only $^{13}C$-sulforaphane was detected. Here, the broccoli seeds were the exclusive $^{13}C$ source.

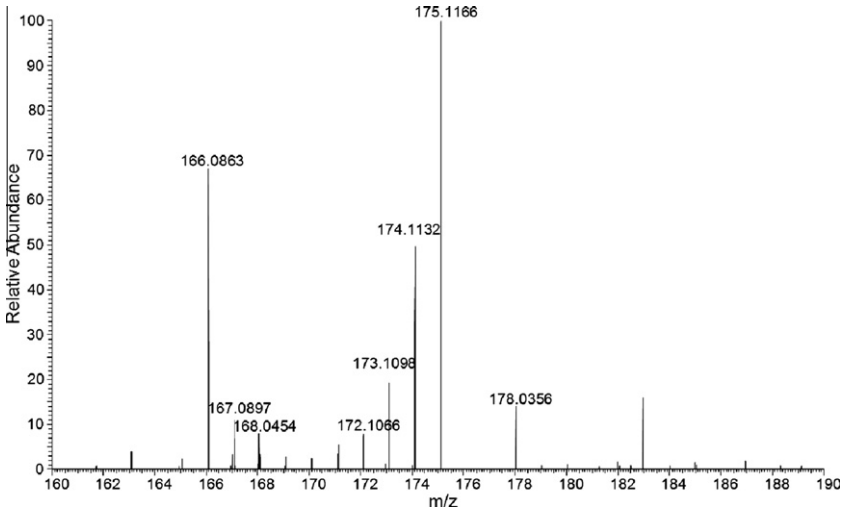


**Fig. 3.** High resolution mass spectrum (*m/z* 160.0–190.0, [$^{12}C_9$- and $^{13}C_9H_{11}NO2 + H^+$] mass 166 and 175) of free phenylalanine in broccoli seedlings grown from $^{12}C$-seeds in a $^{13}C$–$CO_2$ atmosphere.

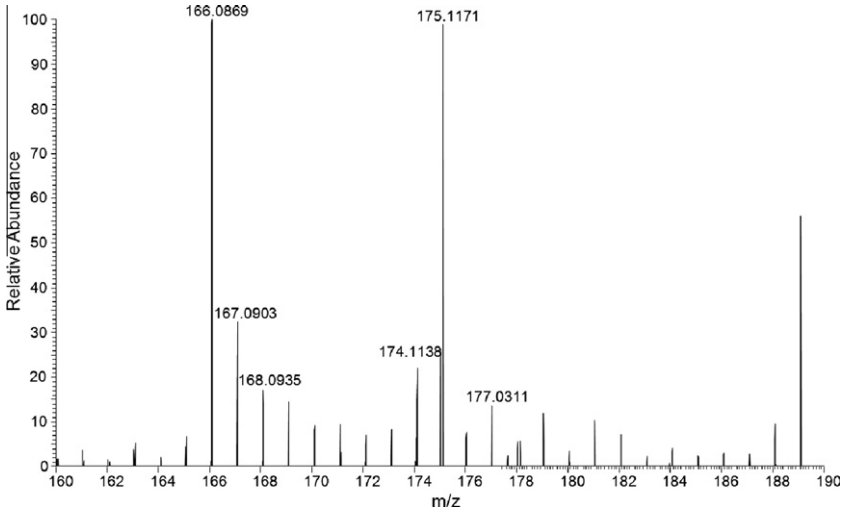


**Fig. 4.** High resolution mass spectrum (*m/z* 160.0–190.0, [$^{12}C_9$- and $^{13}C_9H_{11}NO_2 + H^+$] mass 166 and 175) of free phenylalanine in broccoli seedlings grown from $^{13}C$-seeds in a $^{12}C$–$CO_2$ atmosphere.

**Table 2**
Contrasting labelling data for sulforaphane (Figs. 1 and 2) *vs* a metabolite with high turnover, i.e. phenylalanine in the soluble fraction (Figs. 3 and 4), in two labelling experiments ($n = 2$) showing *de novo* biosynthesis of phenylalanine but not of sulforaphane (n.d.: not detectable).

| Metabolite | Experiment 1 $^{12}C$ seed germinating in $^{13}CO_2$ | | Experiment 2 $^{13}C$ seed germinating in $^{12}CO_2$ | |
|---|---|---|---|---|
| | $^{12}C$-form (pre-germination) Atom% $^{13}C$ | $^{13}C$-form (post-germination) | $^{13}C$-form (pre-germination) Atom% $^{13}C$ | $^{12}C$-form (post-germination) |
| Sulforaphane | (Fig. 1) 1.1 | n.d. | (Fig. 2) 98.2 | n.d. |
| Phenylalanine | (Fig. 3) 2.6 | 94.7 | (Fig. 4) 97.2 | 3.2 |

Since another metabolite, the amino acid phenylalanine in the soluble fraction, was clearly newly synthesised in the broccoli seedlings in both experiments (Table 2; Figs. 3 and 4), our results unambiguously confirm the observation by Bennett et al. (1995) and now directly prove that the sulforaphane in the cotyledons solely originates from seed sulforaphane reserves. Thus, no *de novo* sulforaphane biosynthesis was found here in broccoli seedlings.

The decline in sulforaphane concentration in the cotyledons during the first 14 days after germination will probably result from dilution caused by tissue expansion. It is still unclear why Pérez-Balibrea et al. (2008) found a higher sulforaphane concentration in broccoli seedlings grown in light compared with seedlings grown in the dark, since our results provide evidence that this difference does not result from new sulforaphane biosynthesis under light conditions. Accelerated degradation of sulforaphane in the dark is an alternative hypothesis. We confirm the conclusion of Nakagawa et al. (2006), that consumption of younger broccoli seedlings may be recommended above older seedlings with regard to health aspects and optimal dosage, since they contribute to a higher intake of protective glucosinolates (Rychlik & Adam, 2008).

Since sulforaphane has been reported to induce inhibitory effects on *Helicobacter pylori*-induced stomach cancer (Yanaka

et al., 2009), pulmonary metastasis (Singh et al., 2009), and prostate cancer (Clarke & Dashwood, 2009; Traka et al., 2008), a sulforaphane-enriched diet may help in reducing the occurrence of several forms of cancer in humans (Herra & Büchler, 2010). To further support this conclusion, more knowledge is needed about bioavailability and absorption, distribution, metabolism, and excretion of sulforaphane from broccoli in humans. To study these processes in human clinical studies, we now have developed a strategy for optimal labelling of sulforaphane in broccoli with $^{13}C$.

## Acknowledgements

The authors thank Mr. E. Koning and Mr. R.J.P. Baan (Koppert Cress BV) for kindly providing seeds of *Brassica oleracea* L. from which the commercial product Broccocress® is grown and Mr. J.E. van Doorn (HZPC Holland BV) for useful discussions. The Dutch Innovation Program Food & Nutrition Delta is acknowledged for financial support (Project FND09002).

## References

Bennett, R., Ludwig-Muller, J., Kiddle, G., Hilgenberg, W., & Wallsgrove, R. (1995). Developmental regulation of aldoxime formation in seedlings and mature plants of Chinese cabbage (*Brassica campestris* spp. Pekinensis) and oilseed rape (*Brassica napus*): Glucosinolate and IAA biosynthetic enzymes. *Planta, 196*, 239–244.

Clarke, H. E., & Dashwood, J. D. (2009). Dietary sulforaphane, a histone deacetylase inhibitor for cancer prevention. *Journal for Nutrition, 139*, 2393–2396.

Colditz, G. A., Branch, L. G., Lipnick, R. J., Willett, W. C., Rosner, B., Posner, P. M., et al. (1985). Increased green and yellow vegetable intake and lowered cancer deaths in an elderly people population. *American Journal of Clinical Nutrition, 41*, 32–36.

Fenwick, G. R., Heany, R. K., & Mullin, W. J. (1983). Glucosinolates and their breakdown products in food and food plants. *CRC Critical Reviews of Food Science and Nutrition, 18*, 123–201.

Gorissen, A., Kuikman, P. J., Van Ginkel, J. H., Van, H., de Beek, H., & Jansen, A. G. (1996). ESPAS – An advanced phytotron for measuring carbon dynamics in a whole plant–soil system. *Plant and Soil, 179*, 81–87.

Hellerstein, M. K., & Neese, R. A. (1999). Mass isotopomer distribution analysis at eight years: Theoretical, analytic, and experimental considerations. *American Journal of Physiology, Endocrinology and Metabolism, 276*, 1146–1170.

Herra, I., & Büchler, M. W. (2010). Dietary constituents of broccoli and other cruciferous vegetables: Implications for prevention and therapy of cancer. *Cancer Treatment Reviews, 36*, 377–383.

Jennings, M. E., II, & Matthews, D. E. (2005). Determination of complex isotopomer patterns in isotopically labelled compounds by mass spectrometry. *Analytical Chemistry, 77*, 6435–6444.

Koritsas, V. M., Lewis, J. A., & Fenwick, G. R. (1991). Glucosinolate response of oil seed rape, mustard and kale to mechanical wounding and infestation by cabbage stem flee beetle (*Psyliodes chysocephala*). *Annals of Applied Biology, 118*, 209–221.

Li, Y. C., Kiddle, G., Bennett, R., Doughty, K., & Wallsgrove, R. (1999). Variation in the glucosinolate content of vegetative tissues of Chinese lines of *Brassica napus* L.. *Annals of Applied Biology, 134*, 131–136.

Lim, S., Lee, J., & Kim, J-. K. (2009). Analysis of isothiocyanates in newly generated vegetables, Baemuchea (xBrassicoraphanus) as affected by growth. *International Journal of Food Science & Technology, 44*, 1401–1407.

Mithen, R. (1992). Leaf glucosinolate profiles and their relationship to pest and disease resistance in oilseed rape. *Euphytica, 63*, 71–83.

Nakagawa, K., Umeda, T., Higuchi, O., Tsuzuki, T., Suzuki, T., & Miyazawa, T. (2006). Evaporative light-scattering analysis of sulforaphane in broccoli samples: Quality of broccoli products regarding sulforaphane contents. *Journal of Agricultural and Food Chemistry, 54*, 2479–2483.

Pérez-Balibrea, S., Moreno, D. A., & García-Viguera, C. (2008). Influence of light on health-promoting phytochemicals of broccoli sprouts. *Journal of the Science of Food and Agriculture, 88*, 904–910.

Porter, A. J. R., Morton, A. M., Kiddle, G., Doughty, K. J., & Wallsgrove, R. M. (1991). Variation in the glucosinolate content of oilseed rape (*Brassica napus* L.) leaves. I. Effect of leaf age and position. *Annals of Applied Biology, 118*, 461–467.

Rychlik, M., & Adam, S. T. (2008). Glucosinolate and folate content in sprouted broccoli seeds. *European Food Research Technology, 226*, 1057–1064.

Singh, S. V., Warin, R., Xiao, D., Powolny, A. A., Stan, S. D., Arlotti, J. A., et al. (2009). Sulforaphane inhibits prostate carcinogenesis and pulmonary metastasis in TRAMP mice in association with increased cytotoxicity of natural killer cells. *Cancer Research, 69*, 2117–2125.

Sivakumar, G., Aliboni, A., & Bacchetta, L. (2007). HPLC screening of anti-cancer sulforaphane from important European Brassica species. *Food Chemistry, 104*, 1761–1764.

Steiner, A. A. (1984). The universal nutrient solution. In: Proceedings of the Sixth International Congress on Soilless Culture (pp. 633–649). Wageningen, The Netherlands: International Society for Soilless Culture.

Traka, M., Gasper, A.V., Melchini, A., Bacon, J. R., Needs, P. W. Frost, V., Chantry, A., Jones, A. M. E., Ortori, C. A., Barrett, D. A., Ball, R. Y., Mills, R. D, & Mithen, R. F. (2008). Broccoli consumption interacts with GSTM1 to perturb oncogenic signalling pathways in the prostate. PLoS ONE 3(7), e2568. doi: 10.1371/journal.pone.0002568.

Yanaka, A., Fahey, J. W., Fukumoto, A., Nakayama, M., Inoue, S., Zhang, S., et al. (2009). Dietary sulforaphane-rich broccoli sprouts reduce colonization and attenuate gastritis in *Helicobacter pylori*-infected mice and humans. *Cancer Prevention Research, 2*, 353–360.

Zhang, Y., Talalay, P., Cho, C. G., & Possner, G. H. (1992). A major inducer of anticarcinogenetic protective enzymes from broccoli: Isolation and elucidation of structure. *Proceedings of the National Academy of Sciences of the United States of America, 89*, 2399–2403.