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Glucosinolate profiling of seeds and sprouts of *B. oleracea* varieties used for food

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Abstract

Consumption of plants of the species *Brassica oleracea* is related to a decreased incidence of certain cancer forms in humans, and this has been linked to the presence of glucosinolates in those vegetables. After ripe seeds, sprouts of some brassicaceous plants contain the highest concentration of these compounds and are therefore a good source of glucosinolates for chemoprotection. In the present experiments, the content and distribution of glucosinolates was determined in ripe seeds and sprouts (seedlings) of five varieties of *B. oleracea* (white cabbage, red cabbage, Savoy cabbage, broccoli and cauliflower) by high performance liquid chromatography. The type and concentration of individual glucosinolates varied according to variety of *B. oleracea*, plant parts in which they occurred and the sprouting period of the seed. Concentration of alkyl glucosinolates decreased whereas that of indol-3-ylmethylglucosinolates increased throughout the sprouting period. Roots had the highest glucosinolate concentration in four and seven day old sprouts whereas at both sprouting times, cotyledons had the highest concentration of alkylthio- and alkylsulphinylglucosinolates.

Keywords: Glucosinolates; Sprouts; Glucoraphanin; Indol-3-ylmethylglucosinolates; Chemoprotection

1. Introduction

A number of epidemiological studies have shown in the past years that tumour formation and incidence of cardiovascular diseases are inversely related to the intake of fruit and vegetables (Bao and Fenwick, 2004; Verhoeven et al., 1997). One group of vegetables that has been widely approved for its beneficial effects on human health is that of the brassicaceous vegetables, which contain high concentration of vitamins, minerals and a special group of phytochemicals termed glucosinolates (Ettlinger and Kjær, 1968; Fenwick and Heaney, 1983; Moreno et al., 2006). Glucosinolates are a group of allelochemicals characteristic to plants of the order Capparales that co-occur with myrosinase isoenzymes (E.C. 3.2.1.147) (Bellostas et al., 2003; Sørensen, 1990). When glucosinolates and myrosinases come into contact in the presence of water, e.g. during processing, cutting or chewing of the tissue, glucosinolates are transformed into biologically active products (Brown and Morra, 1997; Rosa et al., 1996). These glucosinolate breakdown products have different structures and physico-chemical properties depending upon the parent glucosinolate and the conditions under which the transformation takes place (Bjergegaard et al., 1994).

Amongst glucosinolate hydrolysis products, isothiocyanates have been frequently mentioned in relation to the chemoprotective effect of brassicaceous vegetables (Fahey et al., 2001; Stoewsand, 1995). The prevailing mechanism for the protective activity of these compounds has been considered to be the induction of phase 2 enzymes, which are involved in detoxification of xenobiotic compounds (Talalay et al., 1995). Other health-promoting effects of isothiocyanates are related to their antioxidant properties (Barillari et al., 2005; Fahey and Talalay, 1999), their capacity for inducing apoptosis (programmed cell death) in cancer cells (Kuang and Chen, 2004; Rose et al., 2005), the inhibition of *Helicobacter pylori* (Fahey et al., 2002) or their capacity to attenuate hypertension (Wu et al., 2004). Since the first identification of its anticarcinogenic activity (Zhang et al., 1992), sulphoraphane, the isothiocyanate from glucoraphanin, is still considered as the most potent inducer of phase 2 enzymes described up to date (Fahey and Talalay, 1999). Other isothiocyanates (ITCs), such as iberin (from glucoiberin), phenylethyl ITC (from gluconasturtiin) or prop-2-enyl ITC (from sinigrin) have also been found to be inducers of

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phase 2 enzymes and to have antiproliferative activity (Adesida et al., 1996; Canistro et al., 2004; Staack et al., 1998; Stoewsand, 1995; Wallig et al., 1998). Under certain experimental conditions, crambene, the nitrile derived from epi-progoitrin has shown similar effects to sulphoraphane (Staack et al., 1998). Other glucosinolate hydrolysis products, such as indol-3-ylcarbinol (I3C) and/or its derivatives produced in the hydrolysis of indol-3-ylmethylglucosinolates (Buskov et al., 2000a; Buskov et al., 2000b), show however a dual effect: at the same time as inducing phase 2 enzymes (Vang et al., 1995; Wortelboer et al., 1992) or contributing to cancer cell cycle arrest (Cover et al., 1998), they have also been associated to a higher risk of cancer development due to the induction of phase 1 enzymes, which are responsible for activation of carcinogens (Kim et al., 1997; Lee and Park, 2003; Stoewsand, 1995).

The chemoprotective effects from brassicaceous vegetables have been directly related to their glucosinolate content (Fahey et al., 1997), therefore the determination of the glucosinolate profile of a given brassicaceous vegetable remains a necessary step in the study of its chemoprotective activity. The glucosinolate profiles vary between species, cultivars of a single species and between tissues of a single plant, as well as varying as a result of processing, storage and growth conditions (Bellostas et al., 2007a; Clossais-Besnard and Larher, 1991; Hansen et al., 1995; Rosa et al., 1996; Sang et al., 1984; Sarwar and Kirkegaard, 1998). The concentration of potentially health-beneficial glucosinolates for a given species has been found to be greater in sprouts than in fully grown plants (Fahey et al., 1997). However, studies of the glucosinolate content of the growing sprouts are scarce and much of the literature still focuses upon either fully grown plants of *Brassica oleracea* crops (Charron et al., 2005; Ciska et al., 2000; Kushad et al., 1999) or seeds (Matthaus and Luftmann, 2000; West et al., 2004). The rapid changes in glucosinolate profile that occur during germination and early seedling growth (Brown et al., 2003; Clossais-Besnard and Larher, 1991; Petersen et al., 2002; West et al., 2004) make the duration of the sprouting period to be a particularly relevant factor in maximizing the concentration of the desirable bioactive compounds.

The present study is part of an ongoing work programme directed at revealing the potential health benefits of using *B. oleracea* sprouts in human diets and it is a continuation of previous research on glucosinolates and glucosinolate-derived products (see above). In the present study, we have investigated the glucosinolate profiles of ripe seeds and seedlings of up to four and seven days of age of five varieties of *B. oleracea* commonly used for food, namely *B. oleracea* var. *capitata* (white, red and Savoy cabbage), *B. oleracea* var. *italica* (broccoli) and *B. oleracea* var. *botrytis* (cauliflower).

2. Materials and methods

2.1. Plant material

The five *B. oleracea* varieties investigated were: *B. oleracea* var. *capitata* (white cabbage) cv. Ditmarsker Ega, *B. oleracea* var. *capitata* (red cabbage) cv. Debut, *B. oleracea* var. *capitata* (Savoy cabbage) cv. Vertus, *B. oleracea* var. *italica* (broccoli) cv. Jade Crosse, *B. oleracea* var. *botrytis* (cauliflower) cv. Winner. All seeds were obtained from Ohlsens Enke, Denmark, except for broccoli seeds that were obtained from Svalöf Weibull AB Hammenhög, Sweden.

2.2. Sampling and analysis

Seeds of the *B. oleracea* varieties mentioned above were imbibed in water with aeration for 24 h (termed day zero). The imbibed seeds were then spread in trays on damp filter paper and allowed to germinate at room temperature (25 °C) in the laboratory. After 1, 2, 3, 4 and 7 days, 50 seeds or seedlings of each cultivar were collected and freeze dried. White and red cabbages were only grown until day 4. For other varieties at day 4 and day 7, the sprouts were separated into cotyledons, hypocotyls and roots. The plant material was extracted following procedures described elsewhere and including two glucosinolates as internal standards (Bjerg and Sørensen, 1987; Sørensen et al., 1999). Glucosinolates were analysed by high performance liquid chromatography (HPLC) and identified by available pure reference compounds as established by standard procedures (Bjerg and Sørensen, 1987; Sørensen, 1990).

3. Results and discussion

3.1. Glucosinolates in ripe seeds and seedlings over growth

Total glucosinolate concentration in the ungerminated seeds of the five varieties tested ranged from 93 μmol g DM^{-1} (broccoli) to 120 μmol g DM^{-1} (red cabbage), whilst sprouts had glucosinolate concentrations ranging from 46 μmol g DM^{-1} (Savoy cabbage at day 4) to 142 μmol g DM^{-1} (red cabbage at day 3). From germination to the 4-day-old stage, total glucosinolate concentration decreased in all varieties except for red cabbage, which showed only a slight increase (Fig. 1). From day 4 to day 7, total glucosinolate concentration increased in Savoy cabbage and cauliflower whereas it decreased in broccoli. Biomass (g DM) decreased along growth in all cases except for broccoli, in which, despite the decrease in glucosinolate concentration, an increase in biomass from day 4 to day 7 allowed the total glucosinolate content per plant not to fall more than 40% of the original content of the seed. In agreement with our observations, Brown et al. (2003) reported an increase in glucosinolate concentration during the first hours after seed imbibition in *Arabidopsis thaliana*, whereas later in the growth total glucosinolate concentration decreased. The decrease in glucosinolate concentration during seedling growth has also been reported in other brassicaceous species (Clossais-Besnard and Larher, 1991; McGregor, 1988; Pereira et al., 2002); it has been suggested to be a consequence of selective glucosinolate metabolism as well as dilution of glucosinolate concentration during tissue expansion (Chen and Andreasson, 2001). Active myrosinase expression seems to parallel the active catabolism that occurs in seedlings (James and Rossiter, 1991; Rask et al., 2000).

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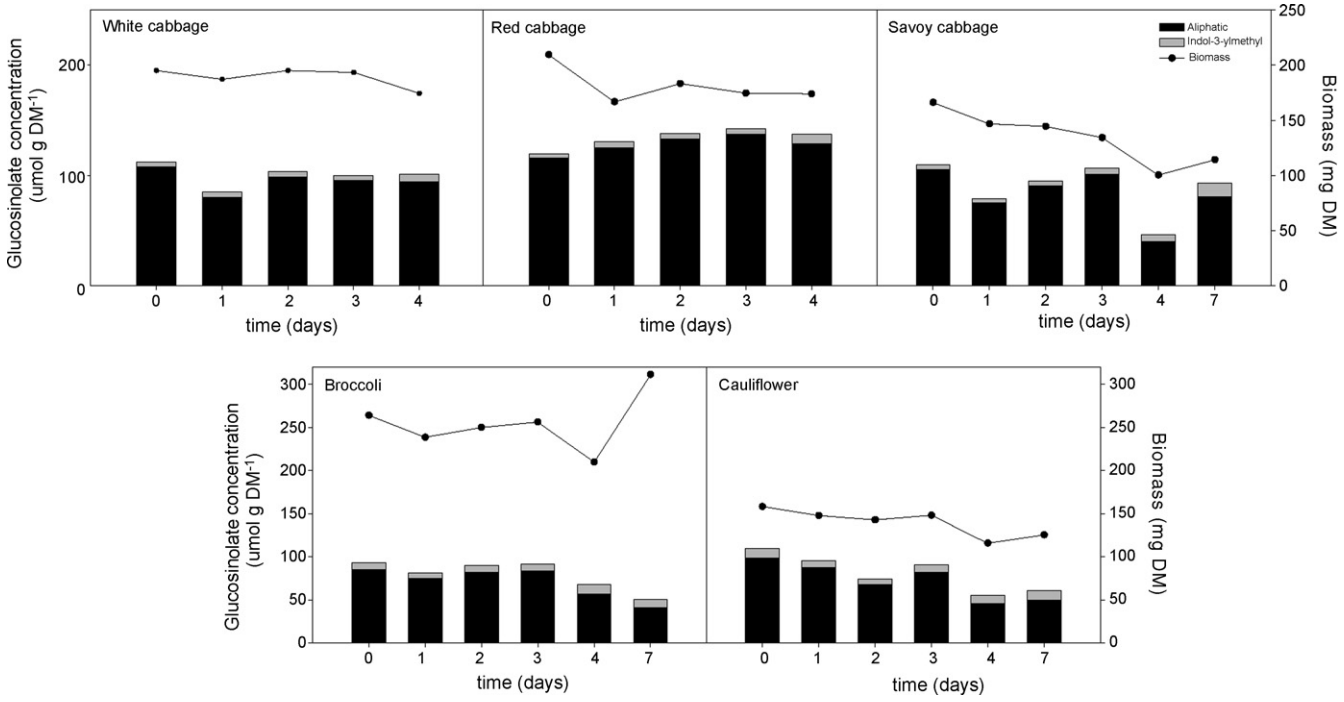

Fig. 1. Evolution of total glucosinolate content (stacked columns on aliphatic and indol-3-ylmethyl) and dry matter of 50 plants over the period studied for the five varieties of *B. oleracea*.

Aliphatic glucosinolates were the major glucosinolate group in seeds of all varieties and remained as such during the growth period monitored. However, their concentration decreased with growth as the concentration of indol-3-ylmethylglucosinolates increased (Fig. 1). The selective catabolism of certain glucosinolates has been previously observed in other species belonging to the order Capparales (Brown et al., 2003; Clossais-Besnard and Larher, 1991; Fieldsend and Milford, 1994; Hopkins et al., 1998) and leads the young seedlings to show a glucosinolate profile intermediate between that of the seeds and the fully developed vegetative tissues (Bellostas et al., 2007b; Brown et al., 2003).

Fahey et al. (1997) have described a decrease in the phase 2 inducer potency (capacity to induce phase 2 enzymes defined as per gram fresh weight) of broccoli with plant age, and this has been related to a decrease in concentration of glucosinolates. In the experiments reported here, maximum glucosinolate concentration in the plant sprouts was achieved at day 3 after germination for all varieties; this coincided with the largest proportion of aliphatic relative to indol-3-ylmethylglucosinolates of all the growth periods monitored (see below).

3.2. Fluctuation of individual glucosinolates along growth – aliphatic versus indol-3-ylmethylglucosinolates

Transformation products from aliphatic glucosinolates have been shown to be particularly potent inducers of phase 2 enzymes (Barillari et al., 2005; Fahey et al., 1997; Staack et al., 1998; Talalay et al., 1995; Zhang et al., 1992) whereas hydrolysis products from indol-3-ylmethylglucosinolates are bifunctional inducers (Bonnesen et al., 1999; Prestera et al., 1993; Vang et al., 1995; Vang et al., 2001). In order to optimize the length of the sprouting period for maximum concentration of a particular group of glucosinolates it is therefore crucial to identify the individual glucosinolates present in brassicaceous sprouts. Details of the individual glucosinolates identified in the five varieties of *B. oleracea* are presented in Table 1.

As reported earlier, the evolution of aliphatic and indol-3-ylmethylglucosinolates throughout growth showed opposite

Table 1
Glucosinolates present in the five varieties of *Brassica oleracea* studied in the present work

Chemical structure of the side chain	Chemical name of side chain	Trivial name
Aliphatic		
	Prop-2-enyl	Sinigrin
	But-3-enyl	Gluconapin
	Pent-4-enyl	Glucobrassicanapin
	(2R)-2-Hydroxybut-3-enyl	Progoitrin
	(2S)-2-Hydroxybut-3-enyl	Epi-progoitrin[a]
	2-Hydroxypent-4-enyl	Napoleiferin
	3-Methylthiopropyl	Glucoibervirin
	4-Methylthiobutyl	Glucoerucin
	(R)-3-Methylsulfinylpropyl	Glucoiberin
	(R)-4-Methylsulfinylbutyl	Glucoraphanin
	(R)-5-Methylsulfinypentyl	Glucoallysin[b]
Aromatic		
	Phenethyl	Gluconasturtiin
Indol-3-yl		
	Indol-3-ylmethyl	Glucobrassicin
	N-Methoxyindol-3-ylmethyl	Neoglucobrassicin
	4-Hydroxyindol-3-ylmethyl	4-Hydroxyglucobrassicin
	4-Methoxyindol-3-ylmethyl	4-Methoxyglucobrassicin

[a] Not present in white cabbage, Savoy cabbage and cauliflower.
[b] Not present in white cabbage and Savoy cabbage.

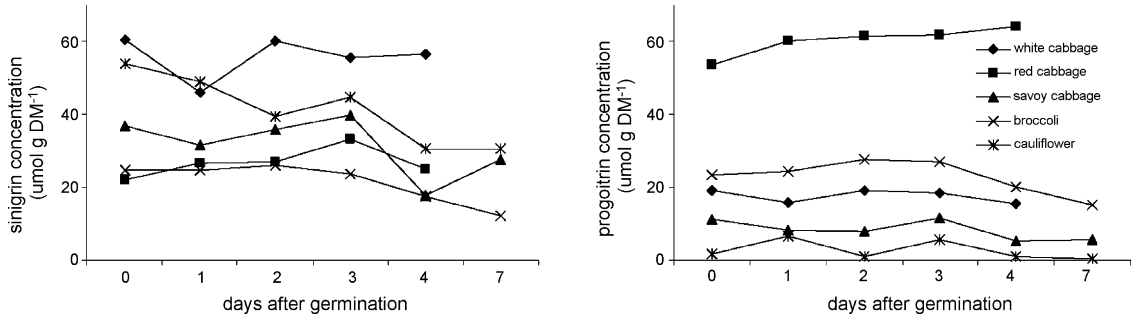

Fig. 2. Evolution of the concentrations of sinigrin (left) and progoitrin (right) from seeds to 7-day seedlings.

trends, the former decreasing with the latter increasing in concentration throughout the sprouting period (Fig. 1). The ratio between the different aliphatic glucosinolates remained more or less constant as growth progressed (Figs. 2–3). However, there was no consistent ranking among the indol-3-ylmethylglucosinolates for any of the five varieties and virtually every moment in growth a different indol-3-ylmethylglucosinolate was the dominant (Fig. 4). The five varieties of *B. oleracea* studied exhibited a distinct profile, with either one or a group of glucosinolates consistently dominating in each variety (Table 2). During the growth period monitored, cauliflower and broccoli had a higher proportion of indol-3-ylmethylglucosinolates than the three cabbage varieties examined, although this difference decreased with time.

Regarding the individual aliphatic glucosinolates, gluconapin concentration was generally highest in seeds (ranging from 0.2 μmol g DM^{-1} in cauliflower to 7.3 μmol g DM^{-1} in red cabbage) and it decreased with growth in all of the varieties of *B. oleracea* studied. Concentrations of napoleiferin, glucobrassicanapin, epiprogoitrin and glucoalyssin were never higher than 1 μmol g DM^{-1}, whereas 3-day seedlings of red cabbage showed the maximum concentration of gluconasturtiin (1.4 μmol g DM^{-1}). Sinigrin (Fig. 2) has already been reported as the major glucosinolate in edible cabbage and cauliflower heads (Bradshaw et al., 1984; Charron et al., 2005; Kushad et al., 1999; Kushad et al., 2004; Sang et al., 1984), seeds and seedlings (Rangkadilok et al., 2002), with values ranging from 6 μmol g DM^{-1} (Kushad et al., 1999; Rangkadilok et al., 2002) to 125 μmol g DM^{-1} (Tookey et al., 1980) depending upon the cultivar and the tissue assessed. Yet, cabbage and cauliflower cultivars reported as having progoitrin (Fig. 2) as the dominating glucosinolate are also frequent (Kushad et al., 2004; Tian et al., 2005). In agreement with the results recorded in our experiments, broccoli cultivars with higher levels of sinigrin and progoitrin than those of alkylthio- and alkylsulphinylglucosinolates have also been described, although these cultivars do not seem to be frequent (Kushad et al., 1999; West et al., 2004). Sprouts of most broccoli cultivars studied in recent

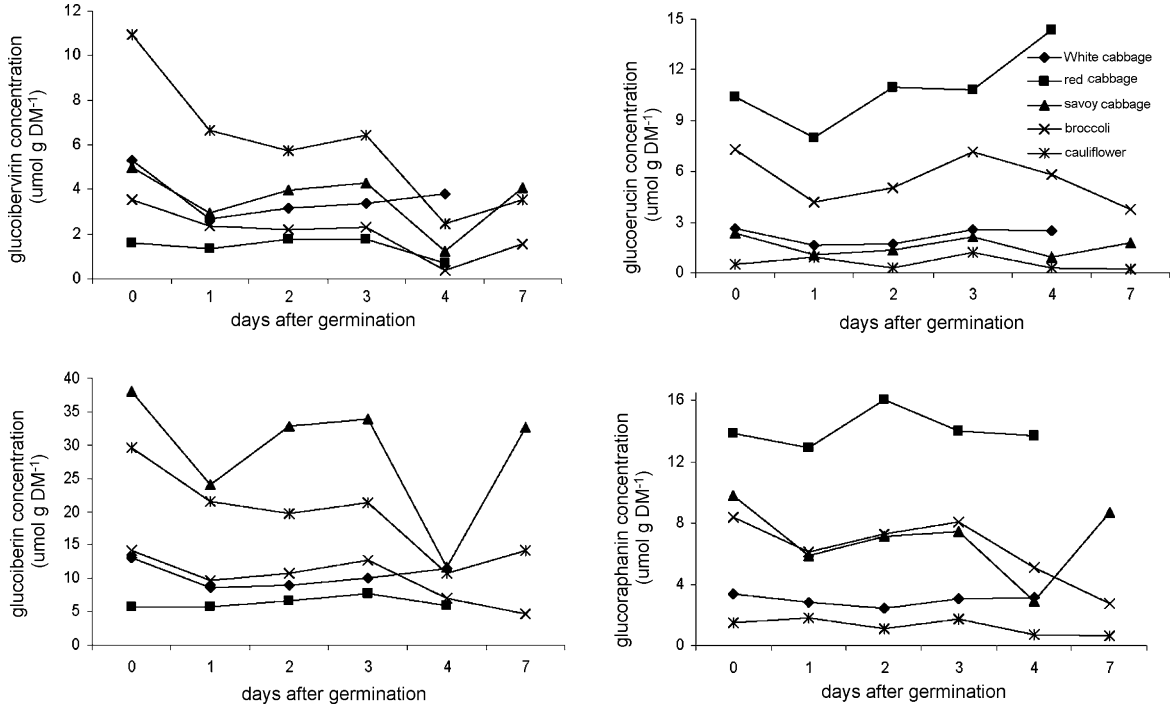

Fig. 3. Evolution of the concentration of the four thiofunctionalized glucosinolates from seed to 7-day old seedlings. Above left: glucoibervirin; above right: glucoerucin; below left: glucoiberin; below right: glucoraphanin.

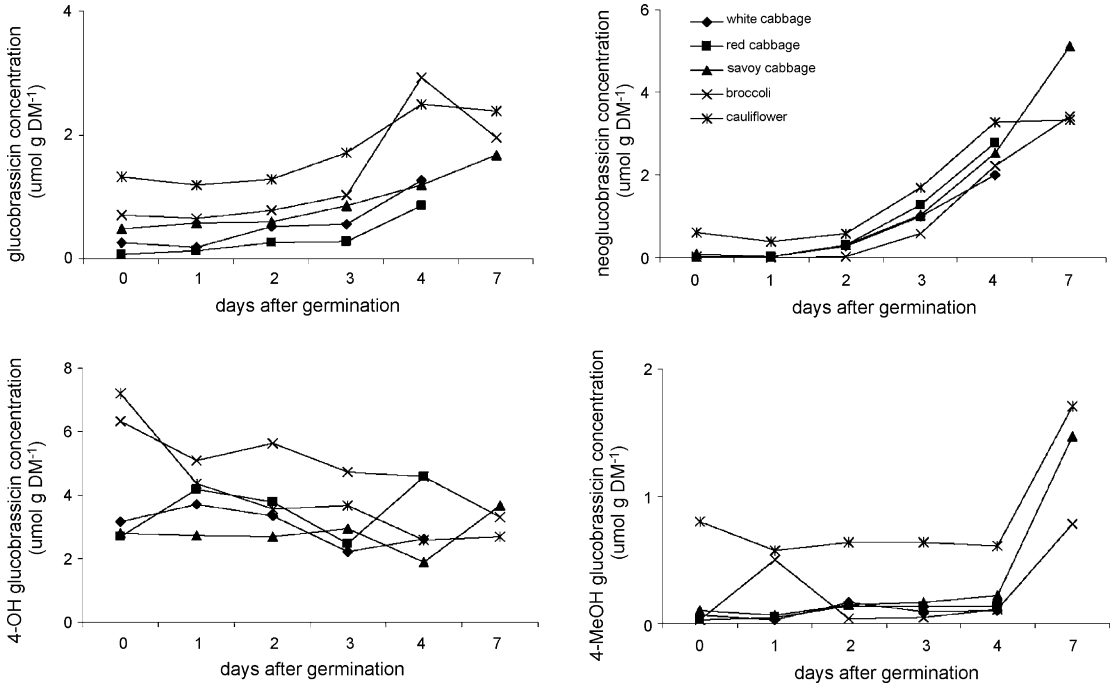

Fig. 4. Evolution of the concentration of the four indol-3-ylmethylglucosinolates from seed to 7-day old seedlings. Above left: glucobrassicin; above right: neoglucobrassicin; below left: 4-OH glucobrassicin; below right: 4-MeOH glucobrassicin.

years contain glucoraphanin as the main thiofunctionalised glucosinolate, with its concentration depending upon genotype and duration of the sprouting period (Charron et al., 2005; Hansen et al., 1995; Kushad et al., 1999; Rangkadilok et al., 2002; Tian et al., 2005; West et al., 2004). In the experiments reported here the concentration of glucoiberin, the alkylsulphinyl-homologue (Table 1) of glucoraphanin, was at all times higher than glucoraphanin itself (Fig. 3). With the exception of red cabbage, glucoiberin was the dominant thiofunctionalised glucosinolate in the varieties studied (Fig. 3). High content of glucoraphanin in red cabbage has previously been shown in fully grown plants (Ciska et al., 2000). A decrease in the concentration of alkylthio- and alkylsulphinylglucosinolates throughout the growth period monitored was observed (Fig. 3). This has been shown previously (Pereira et al., 2002) and it is the reason why young sprouts are preferentially recommended for chemoprotection (Fahey et al., 1997).

In agreement with results reported here, 4-hydroxyglucobrassicin has been found to be the major indol-3-ylmethylglucosinolate in broccoli, cauliflower and cabbage seeds, with varying concentrations depending upon the particular cultivar studied (McGregor, 1988; Sang et al., 1984; West et al., 2004). In the present study, the distribution of individual indol-3-ylmethylglucosinolates among particular tissues was found to follow a trend with glucobrassicin as the dominant indol-3-ylmethylglucosinolate in true leaves and neoglucobrassicin and 4-methoxyglucobrassicin in roots and hypocotyl, as reported elsewhere (McGregor, 1988; Sang et al., 1984). Mature tissues tend to have higher concentrations of indol-3-ylmethylglucosinolates than young tissues (Brown et al., 2003; Fahey et al., 1997; Kushad et al., 1999), which have been related to *de novo* synthesis of this group of glucosinolates with growth (Chen and Andreasson, 2001; Clossais-Besnard and Larher, 1991; Kushad et al., 1999).

3.3. Distribution of the different types of glucosinolates amongst plant parts in young sprouts from the germinating seedling

In order to study the distribution of glucosinolates in the tissues of the seedlings, sprouts were divided into root, hypocotyl and cotyledon sections. Because of the small size of

Table 2
Average content (in percentage) ± standard deviation of different type of glucosinolates along the 7 day sprouting period in the five varieties of *B. oleracea* studied

	White cabbage	Red cabbage	Savoy cabbage	Broccoli	Cauliflower
Sinigrin	56 ± 2	20 ± 2	36 ± 4	27 ± 2	51 ± 2
Progoitrin	17 ± 1	45 ± 1	10 ± 2	29 ± 2	3 ± 3[a]
Alkylthio- and alkylsulphinyl-	19 ± 2	25 ± 2	45 ± 6	30 ± 4	33 ± 5
Indols	4 ± 1	4 ± 1	7 ± 5[b]	10 ± 5	11 ± 4
Residual	4	6	2	4	2

[a] The percentage ranged between 0.7 and 6.9.
[b] The percentage ranged between 3.1 and 12.8.

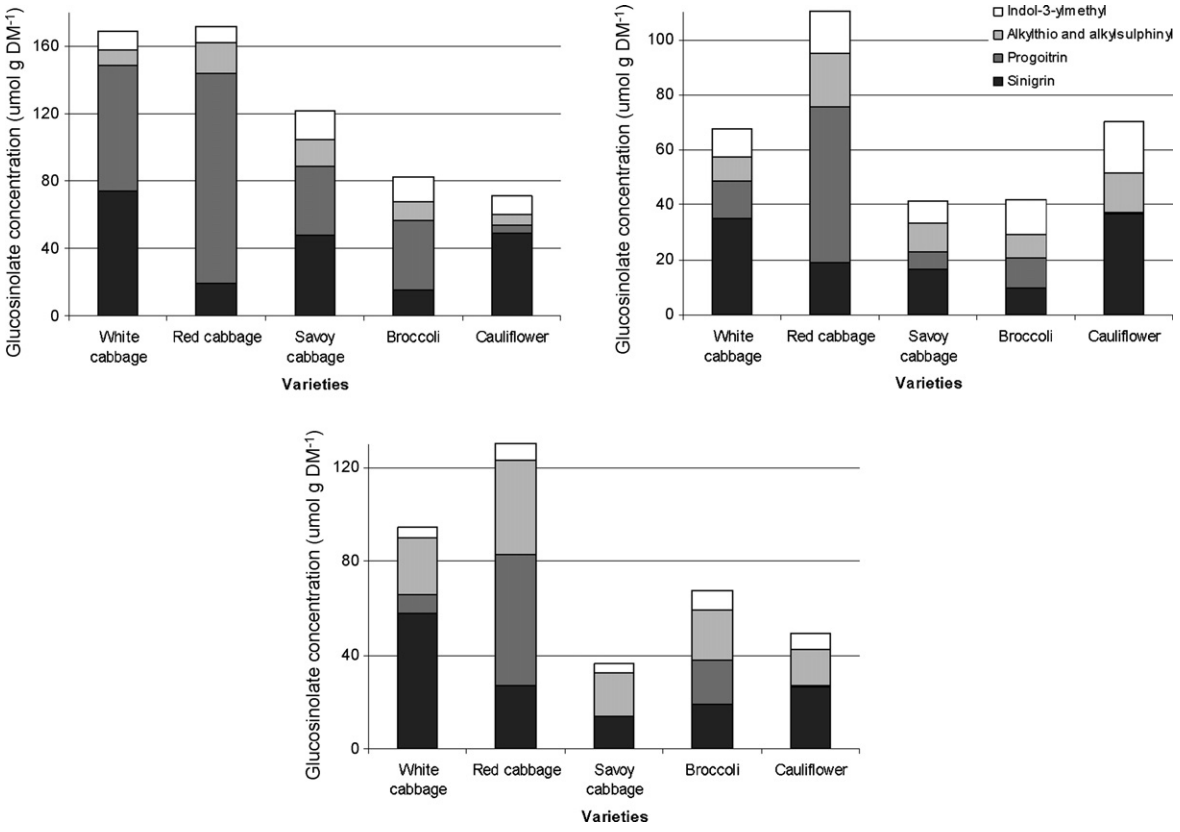

Fig. 5. Distribution of the different glucosinolates in roots (above left), hypocotyl (above right) and cotyledons (below) at day 4.

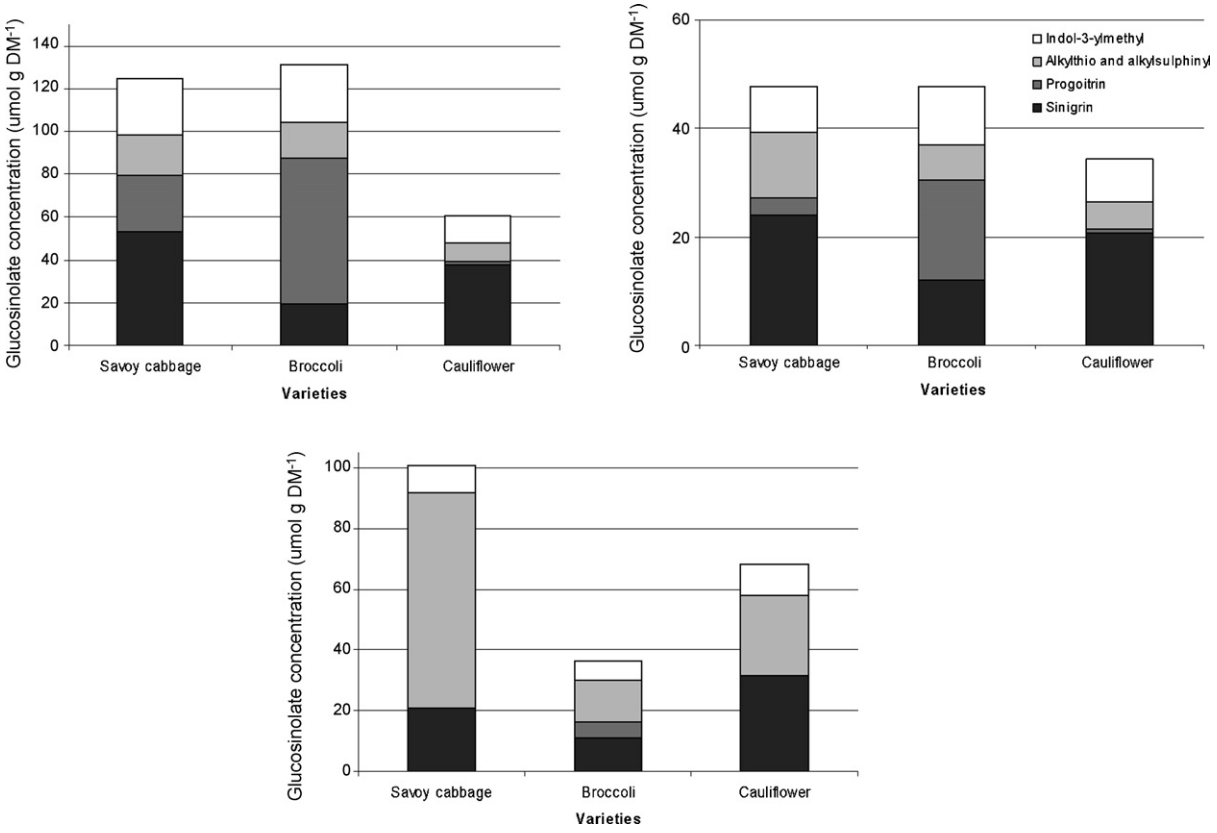

Fig. 6. Distribution of the different glucosinolates in roots (above left), hypocotyl (above right) and cotyledons (below) at day 7.

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Table 3
Relation between the concentration of the major thiofunctionalized glucosinolates in cotyledons of 4-day-old seedlings and seeds

	White cabbage	Red cabbage	Savoy cabbage	Broccoli	Cauliflower
Glucoibervirin	0.70	0.21	0.04	0.03	0.07
Glucoerucin	1.03	1.46	0.30	0.83	0.53
Glucoiberin	1.09	1.30	0.36	0.62	0.45
Glucoraphanin	1.10	1.23	0.34	0.73	0.57
Average	0.98	1.05	0.26	0.55	0.41

Values are expressed as concentration in cotyledons/concentration in seeds and are an estimation of catabolism (value < 1) or anabolism (value > 1).

the seedlings up to 3 days after germination, this operation was performed only at days 4 and 7. In almost all varieties, roots had the highest glucosinolate concentration among the plant organs both in 4- and 7-day-old sprouts. The changes in total glucosinolate concentration in hypocotyl and cotyledons between day 4- and 7 were different depending upon the variety (Figs. 5 and 6). At day 4 and for all varieties, the highest concentration of alkylthio- and alkylsulphinylglucosinolates was observed in cotyledons, whereas indol-3-ylmethylglucosinolates were generally more abundant in the roots and the hypocotyl. Progoitrin was mainly present in roots whereas sinigrin was distributed differently amongst plant parts depending upon cultivar (Fig. 5). Seven-day-old sprouts of all varieties showed a similar distribution pattern, with the dominance of alkylthio- and alkylsulphinylglucosinolates in cotyledons being even more evident (Fig. 6).

The preferential accumulation of indol-3-ylmethylglucosinolates in roots has been previously observed in seedlings of *B. oleracea* (Rosa, 1997) and it has been hypothesized that it is a consequence of the synthesis of this type of glucosinolates taking place in the roots (Rosa, 1997). Accumulation of thiofunctionalized glucosinolates in cotyledons has been previously reported in *A. thaliana,* as a consequence of the seed glucosinolates being retained in the cotyledons (Petersen et al., 2002). In the present experiments, no direct correlation between glucosinolate profile of seeds and cotyledons could be established. However, different metabolic patterns could be observed for alkylthio- and alkylsulphinylglucosinolates depending upon the variety of *B. oleracea* concerned (Table 3). The dynamic changes of glucosinolate levels in any particular tissue depend upon regulation of *de novo* biosynthesis, degradation and mobilization of glucosinolates (Chen and Andreasson, 2001), with the capacity for the *de novo* synthesis varying according to the type of tissue concerned. In general, biosynthetic activities are high in young leaves, shoots and seed pod walls and decrease as the tissue matures (Chen and Andreasson, 2001; Clossais-Besnard and Larher, 1991). Active side chain modification and interconversion of existing glucosinolates have been recorded in cotyledons (Magrath and Mithen, 1993). This could explain the different metabolic patterns of alkylthio- versus alkylsulphinylglucosinolates observed in the different varieties in the experiments reported here.

Despite not having the highest glucosinolate concentration, cotyledons had the highest biomass relative to other tissues; therefore they had the highest total glucosinolate content (expressed as μmol per plant part) of the seedling both at 4 and 7 days. Glucosinolates are plant defence compounds and consistent with this function are accumulated preferentially in the organs that contribute most to plant fitness at a particular moment in the growth cycle (Brown et al., 2003; Grubb and Abel, 2006; Halkier and Gershenzon, 2006). A higher phase 2 inducer potency of aerial organs as compared with roots has already been reported for broccoli sprouts (Pereira et al., 2002). This highlights the importance of considering the distribution of the different type of glucosinolates within the sprout in the production and commercialization of brassicaceous sprouts.

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