Large Scale Determination of Glucosinolates in Brussels Sprouts Samples after Degradation of Endogenous Glucose

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A method was developed for the determination of the glucosinolate content in glucose-rich samples of Brassica vegetables such as Brussels sprouts. Glucose in the samples was enzymatically degraded by the enzyme glucose oxidase (GOD). The resulting hydrogen peroxide and the enzyme GOD were thereafter respectively dissociated and inactivated by a heat treatment at 100 °C. After the degradation of endogenous glucose the glucosinolates were converted into glucose and related metabolites with the enzyme thioglucosidase originating from Brussels sprouts seeds. Glucose released was determined enzymatically with a glucose oxidase/peroxidase assay as a measure for the glucosinolate content of samples. The method was used to study the influence of harvest time, crop production location, and the choice of parental lines on the glucosinolate content of Brussels sprouts F1-hybrids. The sum of sinigrin and progoitrin of F1-hybrids was found to be significantly correlated to the glucosinolate content.

Keywords: *Glucosinolate content; Brussels sprouts; glucose-containing samples*

INTRODUCTION

Glucosinolates are thioglycosides which are present in more than 90 forms in cruciferous species (McGregor et al., 1983). In Brussels sprouts, a high content of the glucosinolates sinigrin and/or progoitrin (Fenwick et al., 1983; Griffiths and Fenwick, 1984) is correlated with bitterness and a low preference score by consumers (Van Doorn et al., 1998a).

Therefore, breeding for a low content of both glucosinolates in Brussels sprouts is preferred for the development of cultivars with a satisfactory flavor for consumers. In recent years, specific ELISA assays have been developed for the determination of sinigrin and progoitrin in Brussels sprouts (Van Doorn et al., 1998b). These assays were applied to support selection for a low sinigrin and progoitrin content in parental lines, inbred populations, and (experimental) F1-hybrids. Newly developed assays, such as ELISAs, have to be compared with the available reference methods (McGregor et al., 1983) to check their reliability, accuracy, and sensitivity. Individual intact glucosinolates are determined routinely by means of HPLC (Bjorkqvist and Hase, 1988). The total glucosinolate content of samples is usually determined via anion exchange chromatography, the subsequent glucose release step with the glucosinolate degrading enzyme thioglucosidase (EC 3.2.3.1), and finally the determination of glucose (VanEtten and Daxenbichler, 1977). The assay for the glucosinolate content is not only an essential reference method for the recovery of glucosinolates but is also relevant for the calculation of the relative content of sinigrin and progoitrin in parental lines and F1-hybrids.

The currently used protocols are too complicated to allow the analysis of large numbers of samples. The time-consuming chromatography step is, however, essential in the currently used method to remove the glucose already present in samples which dominates the released glucose from glucosinolates in the glucose assay.

Various alternative methods have been developed that directly (Thies, 1982) or indirectly (Schnug, 1987) determine the total glucosinolate content of seed samples. The method of Thies, based on complexation of glucosinolates with tetrachloropalladate, is only applicable to seed samples as the matrix of samples from other plant parts of Brassica crops seriously interferes in the assay. The sulfate release method of Schnug also works well on seed samples, but the sulfate release from glucosinolates by the enzyme sulfatase is significantly inhibited at a phosphate concentration higher than 100 mM. The determination of the total glucosinolate content of e.g. seed samples has been further optimized by Smith et al. (1985) and Tholen et al. (1993), who respectively applied an auto analyzer and a TRUBLUGLU meter for relatively large scale determinations. However, seed samples contain a glucose content below 1 g/100 g which does not interfere with the determination of released glucose in the total glucosinolate assay. Therefore, the methods are not applicable on samples of edible parts from the various Brassica species, as these have a sugar content of up to 8 g per kg fresh weight.

In this paper, the glucose degradation of samples with a high glucose content and the subsequent determination of the glucosinolate content is presented. The method is applied to study the influence of crop production location, harvest time at specific sites, and the choice of parental lines on the glucosinolate content of Brussels sprouts cultivars.

MATERIALS AND METHODS

Chemicals and Enzymes. Sinigrin, phenol, aminophenazon, lead acetate, barium acetate, and standard reagents (pro analyze quality), which have been used in buffers and organic solvents (purity >99%), were obtained from Sigma Chemical

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Co. (St. Louis, MO). Glucose oxidase (GOD) and peroxidase (POD) were obtained from Boehringer Mannheim (Mannheim, Germany).

Preparation of Samples. Samples were essentially prepared according to the method of van Doorn et al. (1998b) with some modifications. Samples from Brussels sprout lines and F1-hybrids were obtained from various sites of the Novartis Seeds BV breeding program in The Netherlands. In summary, the Brussels sprouts samples were homogenated in duplicate with 4% (v/v) phosphoric acid, filtered on a paper filter, and a clear supernatant was obtained by centrifugation. The supernatant was neutralized with 5 N KOH at pH 7.0, and precipitates were removed by centrifugation. The clear supernatant was heated in a boiling water bath for 10 min and cooled to room temperature. Subsequently, the samples were additionally treated with 0.5 M lead and barium acetate. After centrifugation, the clear samples were stored at −20 °C, ready for the determination of the glucosinolate content. In all experiments presented in this paper, the glucosinolate content of samples was determined within 3 months after sample preparation. Measurements revealed that the glucosinolate content of frozen samples at −20 °C was constant for a period of one year.

Isolation of Thioglucosidase from Brussels Sprout Seeds. Thioglucosidase was, for economic reasons, isolated on a large scale from Brussels sprout seeds according to the method of Smith et al. (1985) with some modifications. Seeds from the Brussels sprout cultivar Lauris (Novartis Seeds BV) were homogenated in an electric coffee mill and defatted with hexane. Twenty grams of flour was vigorously extracted with 100 mL of hexane in triplicate, and the sediments were subsequently left to dry overnight on filter paper. All purification and isolation steps for thioglucosidase were conducted in the cold room at 4 °C. The defatted flour (20 g) was extracted with 100 mL of 30% (v/v) ice-cold acetone for 30 min. The slurry was centrifuged (Sorvall RC-5B, 13000 rpm, 15 min, 4 °C). The pellet obtained was re-extracted with 50 mL of 30% (v/v) of ice-cold acetone under the same conditions. The second supernatant, obtained after centrifugation, was added to the first supernatant, and the acetone concentration was increased dropwise to 70% (v/v). The precipitate was collected by centrifugation (13 000 rpm, 15 min, 4 °C) and dissolved in a small volume of demineralized water (Barnstead Nanopure, Boston, MA). The dissolved protein was transferred to dialysis tubing and dialyzed for 4 h against, respectively, 2 L of 10 g/L NaCl, 5 g/L of NaCl, and water. The dialysis tubing, containing the enzyme thioglucosidase, was centrifuged (13 000 rpm, 15 min, 4 °C) to remove precipitates, lyophilized, and stored at 4 °C in closed storage tubes until utilized in the glucosinolate assay. The yield was about 300 mg of crude thioglucosidase protein. The specific activity of the crude thioglucosidase was determined with sinigrin as substrate prior to its utilization for the glucosinolate determination described later in this section. The lyophilized enzyme was used in the glucosinolate determination at a concentration of 10 mg/mL in 100 mM sodium phosphate buffer pH 7.0. The enzyme solution was centrifuged (Eppendorf table centrifuge 10 000g, room temperature) prior to utilization in the assay. The enzyme solution for the assay was prepared fresh each time to ensure optimal activity.

Determination of Thioglucosidase Activity in the Enzyme Preparation. Thioglucosidase activity in the crude preparation was determined as the rate of glucose released from sinigrin in 100 mM sodium phosphate buffer pH 7.0 by 50 μL of the enzyme stock solution (10 mg/mL) and by 100 μL of, respectively, 1.2 and 2 mM sinigrin per mL assay buffer. Glucose release, as parameter for thioglucosidase activity, was monitored from 0 to 75 min at intervals of 5 min. The thioglucosidase activity assay was conducted in a reaction mixture of 5 mL. Two hundred microliter subsamples were taken from the reaction reservoir. The thioglucosidase activity in the subsamples was directly inactivated by exposure of the samples for a period of 5 min to 100 °C in a water bath. The heated samples were rapidly cooled on ice to room temperature, centrifuged to remove protein precipitations, and determined enzymatically for released glucose. Incubations with the standard sinigrin concentrations in the absence of thioglucosidase served as a control. The glucose content of the samples was determined in a 100 mM sodium phosphate buffer pH 7.0 containing phenol (1 mg/mL), GOD (65 U/mL), POD (5 U/mL), and aminophenazon (15.6 mg/100 mL) according to the method of Boehringer Mannheim (1989). In this assay, glucose is converted into a complex with a red color which can be determined at 520 nm by the use of a microtiter plate reader. Color development was determined 1 h after the start of the glucose assay.

Degradation of Glucose in Glucosinolate Containing Brussels Sprout Samples. The degradation of glucose was conducted in 96-well microtiter plates (Greiner, The Netherlands) in a 100 mM sodium phosphate buffer (pH 7.0) containing 65 U/mL GOD. A volume of 190 μL GOD/phosphate buffer was mixed with a 10 μL sample and incubated for 4 h at room temperature. During the degradation period the plates were continuously mixed on a microplate mixer to ensure that sufficient oxygen was available for the oxidation of glucose by GOD. After 4 h, the microtiter plates were sealed and heated for 10 min in a water bath of 100 °C which was just not boiling. Thus, the formed hydrogen peroxide was dissociated and the enzyme GOD inactivated. After cooling, the glucose-free samples were used for the determination of the glucosinolate content after a centrifugation step for the collection of adhering condensation on the seals.

Determination of the Glucosinolate Content in Brussels Sprout Samples. Ten microliters 10 mg/mL thioglucosidase solution in 100 mM sodium phosphate buffer pH 7.0 was added to glucose-free samples, as prepared according to the previous section. As a blank, counter samples were incubated in absence of enzyme. Calibration solutions (0, 2, 4, 6, 8, and 10 mM sinigrin) were used to check the thioglucosidase activity across a broad concentration range. After mixing, the plates were sealed, and the glucosinolates were degraded overnight at room temperature. The plates were then centrifuged (IEC Centra 4B with microtiterplate adapters, 4000 rpm, 10 min, room temperature) to remove minor precipitations originating from the thioglucosidase solution. Released glucose from glucosinolates was determined in 100 mM sodium phosphate buffer pH = 7.0 containing phenol (1 mg/mL), GOD (65 U/mL), POD (5 U/mL), and aminophenazon (15.6 mg/100 mL), the glucose assay buffer. A volume of 100 μL of thioglucosidase treated sample was added to 100 μL of glucose assay buffer. After mixing, the samples were incubated for 1 h at room temperature in darkness, and red color formation was subsequently determined in a microtiterplate reader (Biotek, U.S.A.) at a wavelength of 520 nm and a reference wavelength of 600 nm. Glucose calibration solutions (100 μL) (0, 0.08, 0.16, 0.24, 0.32, and 0.40 mM) in 100 μL glucose assay buffer were used to calculate the concentration of released glucose from glucosinolates in the samples. In the calculations, an average glucosinolate molecular weight of 457 was used (Carlson et al., 1987). The glucosinolate content of samples was expressed as mg/100 g fresh weight. The glucosinolate content of samples was determined in duplicate, and only duplicate readings differing 0.020 absorbance unit or less were used for calculations.

Glucosinolate Content of Brussels Sprout Lines during Development. To study the influence of sprout button maturity on the glucosinolate content sprouts from 12 lines were harvested at four monthly intervals starting in October 1989. Samples of 200 g per line were collected on October 15, November 15, December 15, and January 15 in the season 1989−1990. Sprout samples were obtained from the assessment trials, as executed by Novartis Seeds BV for commercial F1-hybrids in De Schermer (north Netherlands on loam soil).

Glucosinolate Content of F1-Hybrids at Various Locations. To study the influence of crop production site on the glucosinolate content 30 sprouts F1-hybrids were grown in 1989 in two different trial fields of Novartis Seeds BV in The Netherlands. The F1-hybrids were grown in, respectively, De Schermer (north Netherlands on loam soil) and in Barendrecht (midwest Netherlands on loam soil). Sprouts of each

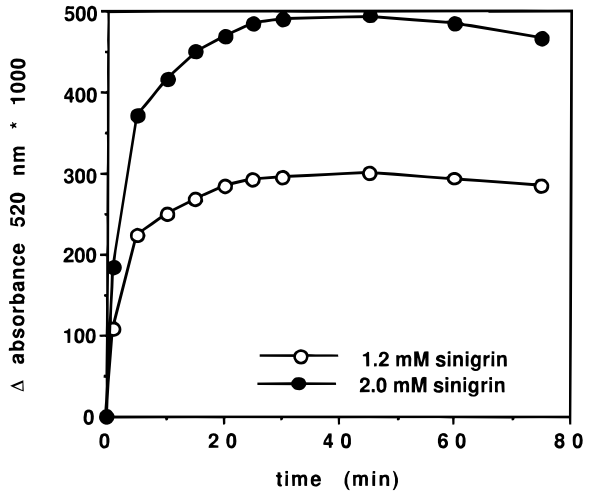

Figure 1. Thioglucosidase activity of a crude enzyme preparation from seeds of the Brussels sprout cultivar Lauris. Glucose release is determined by the GOD/POD assay.

cultivar were harvested simultaneously at the two locations from October to November, the period for an optimal glucosinolate content, and determined for the glucosinolate content. Sprouts of each cultivar were harvested at their optimal harvest time with regard to consumption quality.

Proportion of Sinigrin and Progoitrin in the Glucosinolate Content. The samples of the former paragraph also were determined for the content of sinigrin and progoitrin according to Van Doorn et al. (1998b) to define the proportion of these glucosinolates in the glucosinolate content. The sinigrin and progoitrin content of samples was determined in duplicate, and only duplicate readings differing 0.020 absorbance unit or less were used for calculations.

Glucosinolate Content of F1-Hybrids and Corresponding Parental Lines. In 1989, 150 experimental F1-hybrids and their approximately 50 corresponding parent lines were grown on the trial field of Novartis Seeds BV in De Schermer, in north Netherlands, on loam soil. The F1-hybrids and parental lines were grown under standard conditions, and sprouts of both the hybrids and their corresponding parent lines were harvested at their optimal harvest time with regard to consumption quality and their glucosinolate content determined.

RESULTS AND DISCUSSION

Isolation and Characterization of Thioglucosidase from Brussels Sprouts Seeds. Brassica seeds contain a high content and activity of the enzyme thioglucosidase. The former enzyme can be applied for the determination of the glucosinolate content in Brassica samples by means of glucose release (Smith et al., 1985).

Thioglucosidase was isolated from seeds of the Brussels sprout cultivar Lauris. The thioglucosidase enzyme from Brussels sprout seeds showed a similar behavior in the subsequent isolation steps as reported for the *Brassica napus* enzyme (Smith et al., 1985). The thioglucosidase from Lauris seeds was tested for activity using sinigrin as substrate. Figure 1 shows the rate of glucose release from sinigrin by the enzyme preparation. The enzyme concentration in the assay was chosen to ensure a complete glucose release from glucosinolates within 1 h.

At the specified enzyme concentration, sinigrin was degraded to glucose in about 30 min at both sinigrin concentrations. The observed absorbance values at the end of the activity assay, 0.3 and 0.5 for, respectively, 1.2 and 2.0 mM sinigrin, are equal to the values observed with glucose concentrations of respectively 1.2 and 2 mM in the glucose assay. No glucose release was detected from sinigrin during assay in the absence of thioglucosidase (data not shown). The thioglucosidase preparation was used for the determination of the glucosinolate content with a concentration of 50 μL 10 mg/mL enzyme per mL assay buffer. The thioglucosidase preparation should not contain other glucose producing enzymes such as sucrose invertase, because glucose production via side reactions will lead to an overestimation of the glucosinolate content in samples. The preparation was essentially free from sucrose invertase activity as no degradation of sucrose to glucose and fructose was observed in the presence of the enzyme (data not shown). Tholen et al. (1993) have clearly indicated that Brassica seeds are, apart from thioglucosidase, free from glucose producing enzymes other than sucrose invertase. Their determinations of the seed glucosinolate content by either desulfoglucosinolates and HPLC or by autolysis of glucosinolates with endogenous thioglucosidase, comparable to our method, gave exactly the same results in seven seed samples which varied a 6-fold in glucosinolate content. Brussels sprouts contain, besides glucosinolates and sugars, the glucose containing polymers (hemi)cellulose and starch in amounts that might disturb the glucosinolate assay. The acidic extraction protocol in combination with centrifuge steps at 10.000 $\times$ g excluded the presence of these compounds in samples (van Doorn et al., 1998b).

Degradation of Glucose to Gluconate and Hydrogen Peroxide by GOD. The absence of glucose at the start of the assay is essential for a proper determination of the glucosinolate content of samples via thioglucosidase mediated glucose release. In standard protocols (e.g. VanEtten and Daxenbichler, 1977) samples are freed from sugars by ion-exchange chromatography. Glucosinolates are bound to anion-exchange resins by means of their sulfate group, while glucose and many other compounds have no retention and can easily be washed from the column. The glucosinolates, however, only bind to anion-exchange resins at a relatively low ion strength which means that the extraction of glucosinolates has to be conducted with water, low ionic strength buffers or polar organic solvents such as methanol or ethanol. The extraction of glucosinolates with organic solvents is slow and comprises a sample homogenization step, an extraction step with reflux of the extraction solvent, and finally an evaporation step. In a previous paper, Van Doorn et al. (1998b) have presented a new extraction protocol that allows the extraction of glucosinolates with 4% phosphoric acid on a large scale. Samples prepared with this new protocol contain a phosphate concentration of 350 mM, which is too high for the separation of glucose from glucosinolates by means of ion exchange chromatography. The glucose has to be removed by an alternative method from glucosinolate containing samples in a matrix of 350 mM phosphate. Glucosinolate containing samples can be freed from glucose by its degradation with the enzyme GOD as an alternative to ion exchange chromatography. The enzyme GOD converts glucose to gluconate and hydrogen peroxide, while the glucosinolates remain intact. The oxidation of glucose in glucosinolate containing samples is a nonequilibrium reaction that continues until all glucose is oxidized to gluconate and hydrogen peroxide providing that sufficient oxygen is available (Boehringer Mannheim, 1989). In the glucose degrada-

Table 1. Mean Glucose and Glucosinolate Content of Brussels Sprout Test Samples Used for the Development of the Glucosinolate Assay with Sugar-Containing Samples[a]

sample	glucose (g/100 g)	glucosinolates (mg/100 g)
1	0.6	260
2	1.1	310
3	2.5	190
4	1.4	190
5	1.1	300
6	1.2	300
7	0.7	260
8	0.5	510
9	0.7	320
10	0.6	280

[a] The samples were determined for the content of glucose and glucosinolates in duplicate.

tion step, the resulting hydrogen peroxide can be removed by heating, which simultaneously inactivates the GOD.

The glucose content of 10 test samples, with maximum variation of glucose content, was determined with the GOD/POD assay and varied between 0.5 and 2.5 g/100 g fresh weight (Table 1). A glucose content of 2.5 g/100 g in sprouts corresponds with a glucose concentration of approximately 58 mM glucose in sprout samples as prepared by the method of van Doorn et al. (1998b). In the glucose degradation step, the samples were diluted a further 20 times in GOD/phosphate buffer. The highest glucose concentration (3 mM) was degraded within a period of 4 h. The degradation products in the reaction seemed to have no influence on the conversion of glucose into its degradation products at these concentrations. The power of the degradation protocol is based on the lability of hydrogen peroxide, which is completely degraded into water and oxygen by a heat treatment.

The glucose degradation protocol can be conducted with series of microtiter plates on a much larger scale in comparison to any of the anion exchange protocols (Kuan et al., 1986; McGregor, 1985). An additional advantage of the protocol is that the matrix of the glucose-free samples almost resembles the composition of the buffer which is used in the enzymatic standard protocols for the analysis of glucose (Boehringer Mannheim, 1989; Palmieri et al., 1987).

Glucose Release from Glucosinolates with the Enzyme Thioglucosidase. The overnight degradation of glucosinolates in the 10 glucose-free samples by thioglucosidase resulted in glucose contents which were equivalent to a glucosinolate content between 190 and 510 mg/100 g fresh weight (Table 1). The cultivars differed significantly for the content of glucosinolates (factorial ANOVA, $p = 0.001$, LSD = 57). The values observed are in agreement with, or sometimes even higher than, the presented total glucosinolate figures for Brussels sprout lines and cultivars in the literature (Heaney and Fenwick, 1980; Heaney et al., 1983; Carlson et al., 1987). The glucosinolate assay with an enzymatic glucose degradation step apparently gives realistic glucosinolate figures for samples from sprout lines and F1-hybrids.

The overnight degradation of glucosinolates in glucose free samples was complete, as no intact glucosinolates were detectable by HPLC analysis (data not shown) after the degradation step using the method of Bjorkqvist and Hase (1988). As expected, the glucose and glucosinolate content of the samples were not correlated ($r = 0.57$, $n = 10$, $p > 0.05$).

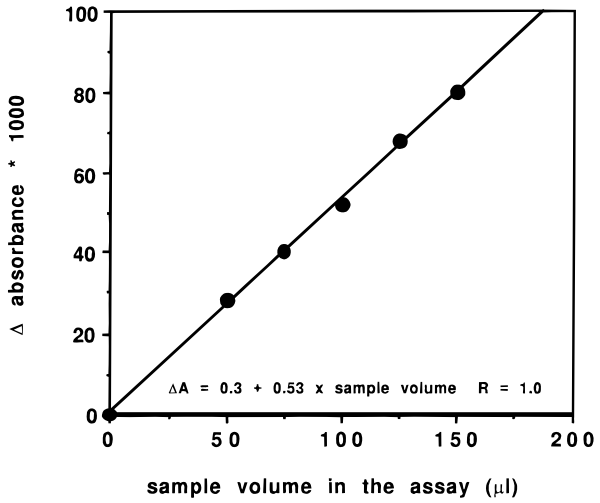

Figure 2. Determination of released glucose from glucosinolates at variable sample volumes in the glucose assay.

Influence of the Sample Matrix on the Glucosinolate Determination. According to the law of Lambert−Beer the absorbance of an ideal enzymatic end-point assay is a log-linear function of the concentration of the analyte. Log-linear relationships between the concentration of analyte and the absorbance can be obtained by variable concentrations of analyte in a fixed matrix or by variable volumes of a matrix with a fixed concentration of analyte. At variable sample volumes in the glucose end-point assay, a linear relationship was observed between the sample volume and the absorbance at the end of the assay (Figure 2). The sample matrix apparently has no influence on the complete conversion of glucose to a red colored complex in the assay. In case of inhibition of the glucose conversion into the red complex by specific factors in the matrix, a typical saturation curve would have been observed between the sample volume and the absorbance values. A sample volume of 100 μL was chosen as a compromise for sample volume, sensitivity, and the maximal absorbance increase in the assay.

Glucosinolate Content of Brussels Sprout Lines during Development. The glucosinolate assay can be used to study the influence of harvest time on the glucosinolate content of Brussels sprout lines and cultivars. The glucosinolate assay was used to study the glucosinolate content of 12 lines in consecutive harvests between October and January. Figure 3 shows the glucosinolate content of a selection of lines and the average content of all lines. Considerable variation in glucosinolate content is observed for consecutive harvests, an indication that the content of cultivars depends on both genetics and the maturity of the sprouts at the time of harvest.

The glucosinolate content of specific lines has an optimum in harvest time. Some lines already start with the highest glucosinolate content in October and decrease in content. Other lines show the highest content in November/December and then decrease in content later. The date of optimum glucosinolate content seems to be correlated with the genetics of the lines. The accumulation of glucosinolates during the development of sprout buttons proceeds until maturity. Overmature sprouts start to reallocate or degrade glucosinolates during the senescence process. On average, the glucos-

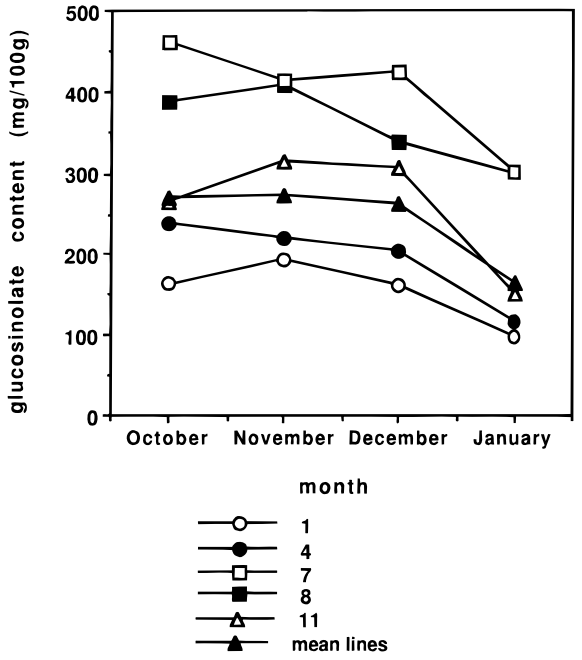

Figure 3. Glucosinolate content of a selection of Brussels sprout cultivars at four consecutive monthly harvests.

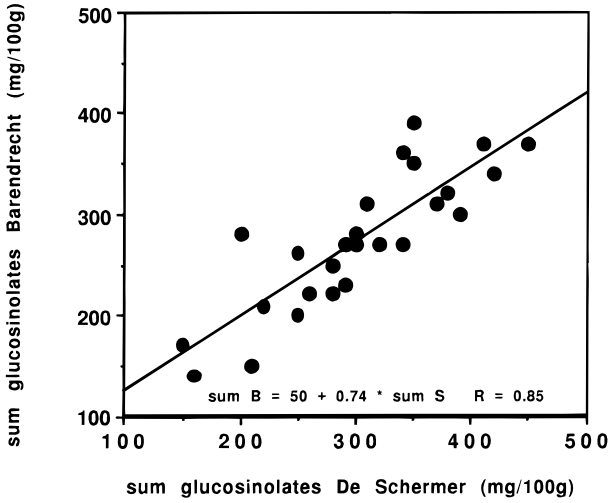

Figure 4. Relationship between the glucosinolate content of 30 F1-hybrids at locations De Schermer (S) and Barendrecht (B).

inolate content of Brussels sprouts cultivars is highest in November. Variations in the sum of glucosinolates as a function of plant age and other developmental parameters is also observed in other Brassicas (McGregor, 1988; Clossais-Besnard and Larher, 1991; Rosa et al., 1994, 1996).

Glucosinolate Content of F1-Hybrids at Various Locations. The glucosinolate content of Brussels sprouts F1-hybrids is relatively stable at different sites an indication that the genotype determines its content for a significant part. Figure 4 shows the relationship between the glucosinolate content of 30 F1-hybrids at location De Schermer and Barendrecht. The glucosinolate content is highly significantly correlated ($r = 0.85$, $p < 0.01$) between the locations. The glucosinolate content in Barendrecht is however significantly lower than in De Schermer (paired two-tail test, $p = 0.001$) indicating that crop production site has an influence on the glucosinolate content of cultivars.

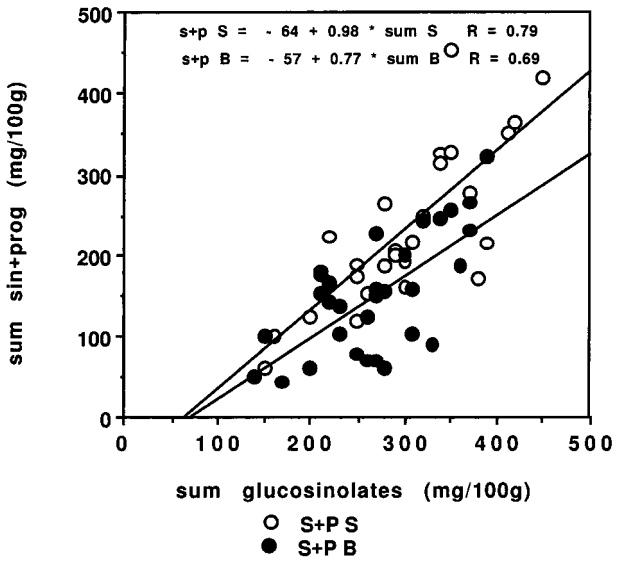

Figure 5. Relationships between the glucosinolate content of 30 F1-hybrids and their sum of sinigrin and progoitrin at the locations De Schermer (S) and Barendrecht (B). The glucosinolate content and the sum of sinigrin and progoitrin are expressed in mg/100 g, s+p and sin+prog denote the sum of sinigrin + progoitrin.

Proportion of Sinigrin and Progoitrin in the Glucosinolate Content. The new method effectively can be used to screen Brussels sprout lines and F1-hybrids for glucosinolate content as a marker for a putative high sinigrin and progoitrin content. A high content of the glucosinolates sinigrin and progoitrin is not accepted by consumers and will result in sprouts with a poor taste (Van Doorn et al., 1998a).

In Figure 5 the relationships are presented between the glucosinolate content of cultivars and their sum of sinigrin and progoitrin at respectively locations De Schermer and Barendrecht. The glucosinolate content of cultivars at both locations is for a significant part comprised of sinigrin and progoitrin (De Schermer: $r = 0.79$, $p < 0.01$; Barendrecht: $r = 0.69$, $p < 0.01$). The glucosinolate content of cultivars is in average for 75 and 55% composed of sinigrin and progoitrin at respectively locations De Schermer and Barendrecht. These results suggest that the glucosinolate determination is a reasonable marker for the sum of these two glucosinolates.

Glucosinolate Content of F1-Hybrids and Corresponding Parental Lines. The glucosinolate content of F1-hybrids is proportional to the content observed in the corresponding parental lines. Figure 6 shows that the glucosinolate content of midparents and the corresponding F1-hybrids is significantly correlated ($r = 0.69$, $p < 0.001$). In Figure 6, considerable deviations between the midparent and F1-hybrid values are observed for many combinations, suggesting that the sum of glucosinolates in the F1-hybrids is only partly related to the sum of glucosinolates of the parental lines.

The new method has proven its accuracy and reliability for the large scale determination of aliphatic glucosinolates such as sinigrin and progoitrin, which are stable during the sample preparation protocol with phosphoric acid (van Doorn et al., 1998b). It was not checked if the method can also be applied for the determination of indole glucosinolates. This is however doubtful, as these are very labile under extreme conditions.

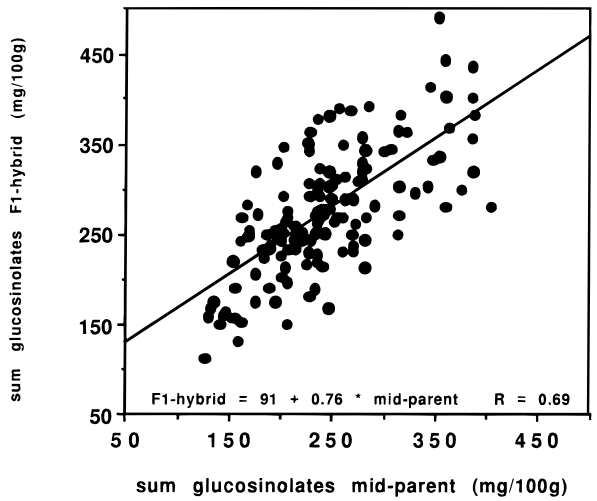

Figure 6. Relationship between the glucosinolate content of the midparent (mean content of the corresponding parental lines of a F1-hybrid) and the F1-hybrid.

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