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ORIGINAL PAPER

Quantitative trait loci analysis of non-enzymatic glucosinolate degradation rates in *Brassica oleracea* during food processing

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Abstract Epidemiological and mechanistic studies show health-promoting effects of glucosinolates and their breakdown products. In literature, differences in non-enzymatic glucosinolate degradation rates during food processing between different vegetables are described, which provide the basis for studying the genetic effects of this trait and breeding vegetables with high glucosinolate retention during food processing. Non-enzymatic glucosinolate degradation, induced by heat, was studied in a publicly available *Brassica oleracea* doubled haploid population. Data were modeled to obtain degradation rate constants that were used as phenotypic traits to perform quantitative trait loci (QTL) mapping. Glucosinolate degradation rate constants were determined for five aliphatic and two indolic glucosinolates. Degradation rates were independent of the initial glucosinolate concentration. Two QTL were identified for the degradation rate of the indolic glucobrassicin and one QTL for the degradation of the aliphatic glucoraphanin, which co-localized with one of the QTL for glucobrassicin. Factors within the plant matrix might influence the degradation of different glucosinolates in different genotypes. In addition to genotypic effects, we demonstrated that growing conditions influenced glucosinolate degradation as well. The study identified QTL for glucosinolate degradation, giving the opportunity to breed vegetables with a high retention of glucosinolates during food processing, although the underlying mechanisms remain unknown.

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Introduction

Glucosinolates (GLs) are an important group of secondary plant metabolites involved in the plant defense system and, from a human point of view, GLs exhibit interesting health-promoting properties. In epidemiological studies, the intake of glucosinolate (GL)-containing vegetables is related to a reduced incidence of certain cancers. Epidemiological data associate the cancer-protective effects of *Brassica* vegetables to GLs; however, no biological activity of intact GLs could be demonstrated in in vitro studies (Traka and Mithen 2009). Upon cell damage, GLs are hydrolyzed by the endogenous enzyme myrosinase (β-thioglucosidase, EC 3.2.1.147), which results in enzymatic degradation and the formation of a variety of breakdown products. One type of breakdown products, isothiocyanates, are bioactive by affecting the process of carcinogenesis in several organs, such as lung, stomach, colorectum and breast (Traka and Mithen 2009). In the absence of active myrosinase in the food ingested, GLs can also be converted into breakdown products by the human gut flora, although this conversion is less efficient (Shapiro et al. 1998, 2001).

The large variation in the content and composition of GLs in *Brassica* vegetables is reviewed extensively by Verkerk et al. (2009). Although this variation is caused by

several factors such as environmental factors, including soil, climate and fertilization, the most important factor determining the GL content is genetic variation (Verkerk et al. 2009). Many genes involved in the GL pathway have been studied using the natural variation combined with molecular marker techniques and subsequent cloning of the identified quantitative trait loci (QTL). Characterization of QTL for GL biosynthesis has been conducted in *Arabidopsis* (Kliebenstein et al. 2001a) and *Brassicaceae* (Feng et al. 2012; Lou et al. 2008). Genes involved in the GL pathway are summarized by Sonderby et al. (2010) and Wang et al. (2011).

The levels and types of GLs in *Brassica* vegetables show natural genetic variation and have been altered further by breeding, indirectly through the selection of taste and flavor and possibly for resistance to herbivores (Verkerk et al. 2009), and directly by breeding for enhanced levels of specific GLs expected to be associated with health benefits. Breeding for a higher GL content in broccoli, in particular glucoraphanin (4-methylsulfinylbutyl-GL), was achieved by introgression of three small segments of the genome of a wild *Brassica* species, *Brassica villosa,* into a broccoli background (Mithen et al. 2003; Sarikamis et al. 2006). Furthermore, it was shown that the high glucoraphanin levels are retained during cooking for short times. As a result of this breeding strategy, broccoli with a high content of glucoraphanin was launched into the UK market in autumn 2011 under the name Beneforte (John Innes Centre News 2012).

Breeding for higher GL concentrations is one way to increase the intake of GLs. However, from a food science perspective, optimizing food processing methods to retain the GLs is equally important, since enhanced levels in primary production can easily be lost during processing and preparation (Dekker et al. 2000). Three main mechanisms of GL losses during food processing have been described: (a) enzymatic breakdown by myrosinase, (b) leaching of GLs and enzymatic breakdown products into the cooking water and (c) thermal degradation of GLs (Dekker et al. 2000). The term “thermal degradation”, as one sub-process, refers to the GL degradation solely induced by heat without myrosinase activity. Myrosinase is a thermolabile enzyme, the activity of which is considerably reduced at moderately high temperatures. A treatment for 3 min at 60 °C reduced the myrosinase activity by 90 % in broccoli (Ludikhuyze et al. 1999), a microwave treatment for 120 s with a final temperature of 88 °C resulted in almost complete loss of myrosinase activity in cabbage (Rungapamestry et al. 2006) and steaming for 6 min led to almost complete loss of myrosinase activity in broccoli reaching a temperature of 80 °C (Verkerk et al. 2010). These studies demonstrate that the temperatures applied during industrial processing and domestic preparation of *Brassica* vegetables quickly inactivate myrosinase, leaving leaching and thermal degradation as the main mechanisms for GL losses in processing and preparation.

A study, comparing the thermal degradation of GLs for five different vegetables, showed that the stability of structurally identical GLs differed in different *Brassica* vegetables (broccoli, Brussels sprouts, red cabbage, Chinese cabbage and Pak Choi). The five studied vegetables differ in many traits, such as metabolic composition, and hence provide different reaction environments for thermal degradation (Dekker et al. 2009). These differences in metabolic composition are determined by biochemical traits and will be (partly) genetically regulated. Therefore, it is hypothesized that thermal degradation of GLs is (partly) genetically regulated and therefore genomic regions can be identified influencing the thermal degradation rates of GLs.

To test this hypothesis, GL thermal degradation was studied in a *Brassica oleracea* doubled haploid population (AGDH, broccoli × Chinese kale, (Bohuon et al. 1996)) using a kinetic modeling approach as described by Hennig et al. (2012b). The values of the obtained degradation rate constants were used as traits to perform QTL analysis. The described QTL analysis of GL degradation provides novel insights into the role of the genetic background during food processing. Observed QTL could facilitate breeding of vegetables with a higher retention of GLs during processing. Furthermore, the future identification of biochemical traits underlying the observed QTL and subsequently genes influencing the processing of vegetables could lead to new breeding strategies for vegetables that retain GLs better than others during food processing. The proposed strategy complements breeding strategies for high GL content, to retain high GL concentrations throughout the entire food production chain and assure a high GL concentration at the stage of consumption.

Materials and methods

Plant material

The doubled haploid (DH) population AGDH, developed by Bohuon et al. (1996), was used to study GL thermal degradation. This population was developed by crossing two DH parents, a rapid-cycling Chinese kale line, *B. oleracea* var. *alboglabra* (A12DHd), and a calabrese broccoli line, *B. oleracea* var. *italica* (GDDH33), through microspore culture of the F_1. Seeds were sown into soil, plants were transplanted into 19 cm diameter pots after 2 weeks, randomized and grown for 6 weeks after transplanting in a greenhouse in Wageningen (The Netherlands). A total of

100 DH lines of the population were grown in five replicates in spring 2009 (end of March till end of May) under natural light and temperature conditions. Due to the lack of seeds, the parental lines were not included in the study. Temperatures ranged from 5 to 16 °C during the night and from 13 to 30 °C during the day. Fertilizer was given two to three times per week (electric conductivity 2.1). A subset of ten DH lines of the population, selected based on their GL degradation in 2009, was grown again in spring 2011 (end of March till end of May) in a greenhouse in Wageningen. Temperatures ranged from 17 to 22 °C (night/day); however, on sunny and warm days the temperature could rise to 30 °C during the afternoon. Artificial light was applied if the natural photoperiod was shorter than 16 h.

Eight weeks after sowing, leaves without petioles were harvested in the morning and transported on ice to the laboratory for further sample preparation. Harvesting of the 100 DH lines in spring 2009 was performed on four consecutive mornings, but all five biological replicates of the same DH line were harvested on the same day. Harvesting in spring 2011 was performed in one morning. All leaves from the five plants per DH line were pooled to prepare one homogenous sample per DH line. This approach was chosen to prepare an average sample accounting for the variation during growth.

To study the GL thermal degradation as a sole mechanism, without enzymatic GL degradation, the myrosinase was inactivated by microwave treatment. Microwave treatment was performed at high power for a short time, which has shown to fully inactivate myrosinase with high retention of GLs (Oerlemans et al. 2006; Verkerk and Dekker 2004). Leaves were cut into pieces of about 3 × 3 cm, of which 75 g was placed into a plastic beaker and held on ice until microwave treatment. In total, five plastic beakers, each containing 75 g of leaves, were placed at the same time in a microwave at 900 W for 6 min. After the microwave treatment, samples were immediately cooled on ice, weight loss was recorded and the samples were subsequently freeze dried. Dried samples were weighed to record the water loss, ground into a fine power and stored at −20 °C until further treatment.

Chemicals

Solvents used for extraction and chromatography were of HPLC grade and bought from Biosolve (Valkenswaard, The Netherlands). The DEAE Sephadex-A25 and sinigrin (prop-2-enyl-GL) were purchased from Sigma-Aldrich (Zwijndrecht, The Netherlands). The internal standard glucotropaeolin (benzyl-GL) was purchased from the Laboratory of Biochemistry, Plant Breeding and Acclimatization Institute at Radzikow, Błonie, Poland. Two different batches of sulfatase from *Helix pomatia* (Sigma-Aldrich, Zwijndrecht, The Netherlands) were used for the experiments (lot no. 089K3797 and 029K3782).

Thermal degradation

Dried plant powder was weighed into 2 ml plastic tubes with screw caps and reconstituted with MilliQ water to obtain 200–220 mg plant material having the same water content as before the microwave treatment and drying. Tubes with reconstituted samples were heated in a heating block at 100 °C for 0, 15, 30 and 60 min. All heating experiments were performed in duplicate. Temperatures were monitored inside the sample with a thermocouple, which was placed through the cap of the tube. The average time to reach 100 °C in the samples amounted to 4 min. After the predefined heating times, samples were cooled on ice and stored at −20 °C till GL analysis.

Glucosinolate analysis

GLs were analyzed as desulfo-glucosinolates by HPLC. The reconstituted and heated samples were extracted with hot methanol (70 %) and subsequently desulfated in microtiter plates with a purified sulfatase as described by Hennig et al. (2012a).

Modeling

Mathematical modeling was used as a tool to describe the thermal degradation speed of GLs in a quantitative way. Since the molecular mechanism of GL degradation is not known, the reaction can be described by empirical models. Equation (1) displays the integral of the general rate law with respect to time for the case that the order of the reaction equals 1 with c the GL concentration at a certain time point, c_0 the GL concentration at time zero (without heating), k_d the degradation rate constant and the heating time t at a constant temperature. For this first-order mechanism, GLs are in one limiting reaction step degraded into other products, whereby the rate is proportional to the concentration of the remaining GLs. In several studies, the first-order equation has been applied to model GL thermal degradation (Dekker et al. 2009; Oerlemans et al. 2006; Oliviero et al. 2012) and it has been shown for the parental lines of the DH population used in this study that GL degradation follows a first-order reaction independently from the growing season (Hennig et al. 2012b).

$$c = c_o \cdot e^{-k_d \cdot t} \quad (1)$$

Statistics

The Shapiro–Wilk normality test was performed using the program R (http://www.r-project.org) to test for the normal

distribution of the data (significance level $p < 0.05$). The Pearson correlation coefficients and the significance of the correlations were determined using the software IBM SPSS Statistics 19. The Bonferroni adjustment was used to correct the significance value for multiple testing by dividing the significance levels $\alpha = 0.05$ and $\alpha = 0.01$ by the number of paired comparisons made. Analysis of variance (ANOVA) was performed to test for an influence of the sampling on the degradation rate constants (k_d) using the software IBM SPSS Statistics 19. No significant influence of the harvesting day on the k_d values was found for glucobrassicin, sinigrin, progoitrin, gluconapin and neoglucobrassicin. Marginal differences were detected for glucoiberin and glucoraphanin.

QTL mapping

The linkage map, used for QTL analysis, has been constructed with a subset of 107 DH lines of the AGDH population. The map was constructed based on restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP) and microsatellite markers published by Sebastian et al. (2000). The final map was constructed with a subset of 107 DH lines using the software JoinMap 4.0. The genetic map constructed with 210 DH lines of the population is publicly available on www.brassicainfo.com. The total length of the two maps are comparable, but the length of the individual linkage groups differ, due to a different number of DH lines used for the map construction in this study.

QTL mapping was performed using the Software MapQTL 6.0 (Van Ooijen 2009) with the GL thermal degradation rate constants (k_d values) and the initial GL concentrations (c_0 values) as phenotypic traits. The analysis started with interval mapping (IM) and a permutation test was applied to each data set (1,000 repetitions) to decide the LOD (logarithm of odds) threshold ($p \leq 0.05$). In this study, a genome-wide LOD value of 2.7 was used as significant threshold for the c_0 value and the k_d value of the GLs glucobrassicin (indol-3-ylmethyl-GL) and glucoraphanin (4-methylsulfinyl-GL); a genome-wide LOD of 2.8 was used as the significant threshold for the c_0 value of glucoiberin (3-methylsulfinylpropyl-GL) and 2.6 for the c_0 value of gluconapin (but-3-enyl-GL). The results of the interval mapping were used to identify putative QTL, which were selected as co-factors if their LOD value exceeded the chromosome-wide threshold. Co-factors were verified by using the automatic co-factor selection tool and only significant markers ($p < 0.02$) were used as co-factors for the subsequent restricted multiple QTL model mapping (RMQM). A map interval of 5 cM was used for both IM and RMQM analyses.

The data were analyzed for epistatic interaction by regression analysis using the software IBM SPSS Statistics 19. The peak markers of each QTL which had low missing values were used to estimate the contributions of individual markers and their interaction to the phenotypic variance. The individual effects and the interactions were fitted into the regression model for the interaction of two QTL (Eq. 2) and a p value <0.05 was applied to determine the significance of the interaction.

$$y = \beta_0 + \beta_1 Q_1 + \beta_2 Q_2 + \beta_k Q_1 \cdot Q_2 \qquad (2)$$

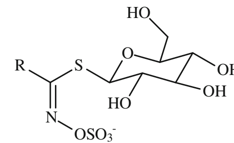
Results

Initial glucosinolate concentrations in the doubled haploid population

The GL profile and concentrations varied substantially throughout the DH population and only few GLs could be identified in all DH lines. The most abundant GLs identified in all of the 100 DH lines of the DH population were the aliphatic GL glucoraphanin (4-methylsulfinylbutyl-GL) and the indolic GL glucobrassicin (indol-3-ylmethyl-GL). Table 1 gives an overview of the range of initial GL concentrations (c_0 values) determined in the DH population and shows the structures of the GLs including the chemical and trivial names. Other aliphatic GLs present in most of the DH lines were glucoiberin (3-methylsulfinylpropyl-GL), progoitrin ((2R)-2-hydroxybut-3-enyl-GL), sinigrin (prop-2-enyl-GL) and gluconapin (but-3-enyl-GL). Furthermore, the indolic GL neoglucobrassicin (*N*-methoxyindol-3-ylmethyl-GL) could be identified in 91 DH lines with the lowest GL concentrations. The histograms of the initial GL concentrations (c_0 values) of glucoraphanin and glucobrassicin are presented Fig. 1a, b. The Shapiro test for normal distribution showed that both data sets were not normally distributed ($p < 0.05$). QTL mapping was conducted for the initial GL concentrations (c_0 values) and the results are presented in Fig. 2a–d. Two QTL were identified for the aliphatic GL glucoraphanin, one on linkage group 9 (30 % explained variation) and the other on linkage group 1 (9.4 % explained variation). The QTL for glucoiberin co-localized with one of the QTL for glucoraphanin on linkage group 9 (28 % explained variation); both GLs are methylsulfinyl-GL differing by only one C-atom in their side chain. On linkage group 7, a small QTL for another aliphatic GL, gluconapin, was located explaining 16 % of the variation. Two QTL for the initial GL concentration of the indolic GL glucobrassicin were located on linkage group 3 (15 and 12 % explained variation).

Table 1 Chemical structure of identified glucosinolates and range of modeled initial glucosinolate concentrations (c_0 values) in the DH population in spring 2009

General glucosinolate structure



Glucosinolate	Structure side chain	DH lines with detected glucosinolate (*N*)	Concentration range [µmol/g FW]	Average [µmol/g FW]
Glucoiberin[a] *3-Methylsulfinylpropyl-*		65	0.011–0.321	0.096
Glucoraphanin[a] *4-Methylsulfinylbutyl-*		100	0.006–1.568	0.277
Sinigrin[a] *Prop-2-enyl-*		40	0.017–0.675	0.169
Gluconapin[a] *But-3-enyl-*		60	0.028–1.180	0.306
Progoitrin[a] *(2R)-2-Hydroxybut-3-enyl-*		45	0.010–1.134	0.360
Glucobrassicin[i] *Indol-3-ylmethyl-*		100	0.008–0.328	0.114
Neoglucobrassicin[b] *N-methoxyindol-3-ylmethyl-*		91	0.004–0.088	0.020

[a] Classified as aliphatic glucosinolate according to the structure of the side chain

[b] Classified as indolic glucosinolate according to the structure of the side chain

Glucosinolate thermal degradation rate constants

The value of the thermal degradation rate constant k_d describes the steepness of the degradation curve and is hence a quantitative measure for the rate of GL degradation (Hennig et al. 2012b). The distribution of GL degradation rate constants (k_d values) of the two most abundant GLs in the DH population is presented in Fig. 3. The average k_d value of glucobrassicin amounted to 3.21×10^{-2} min^{-1} and the average k_d value of glucoraphanin amounted to 1.85×10^{-2} min^{-1}; hence, the degradation of glucobrassicin was almost twice as fast as the degradation of glucoraphanin. For both GLs, the k_d values show a normal distribution throughout the DH population (Shapiro–Wilk normality test, $p > 0.05$). Table 2 gives an overview of the variation of the k_d values for all GLs identified in the DH population. The highest variation was observed for glucoraphanin (8.7-fold difference), whereas the variation of the k_d values for the other GLs was lower (around threefold). The average k_d values were lower for the GLs with an aliphatic side chain (glucoiberin, sinigrin, progoitrin, glucoraphanin, gluconapin) compared to the GLs with an indolic side chain (glucobrassicin, neoglucobrassicin). The data show that GL degradation is dependent on two factors: (a) the structure of the GL side chain and (b) on the plant matrix, as illustrated by the distribution of the GL degradation throughout the DH population. Furthermore, the low correlation coefficients between the k_d and the c_0 values (Table 2) show that the GL degradation is not dependent on the initial GL concentration.

Correlation of thermal degradation of different glucosinolates

As shown in Table 1, the GL concentrations varied throughout the DH population and not all GLs could be identified in all the DH lines. To determine if the degradation of different GLs in the same DH lines are correlated,

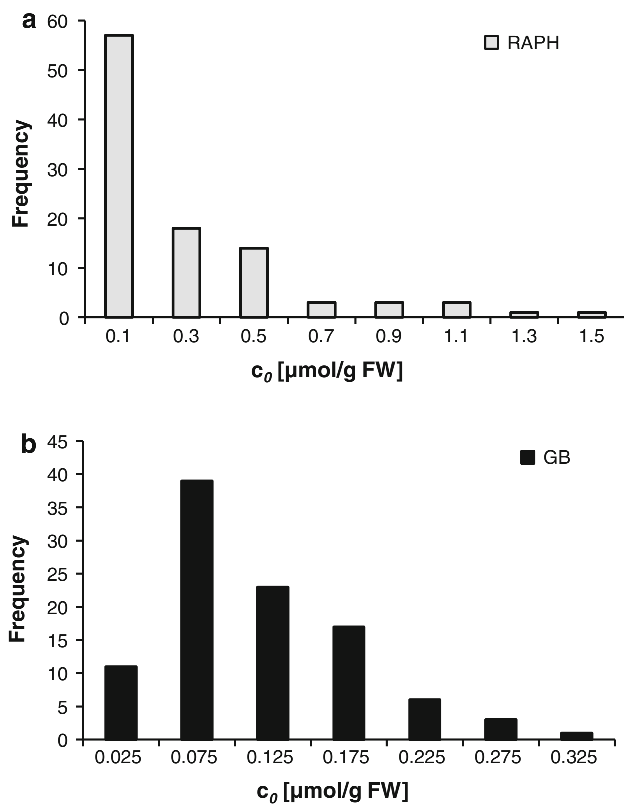


Fig. 1 Histogram of the initial glucoraphanin (**a**, RAPH) and glucobrassicin (**b**, GB) concentrations (c_0 values) in the DH population in spring 2009. *Bars* represent the observed frequencies

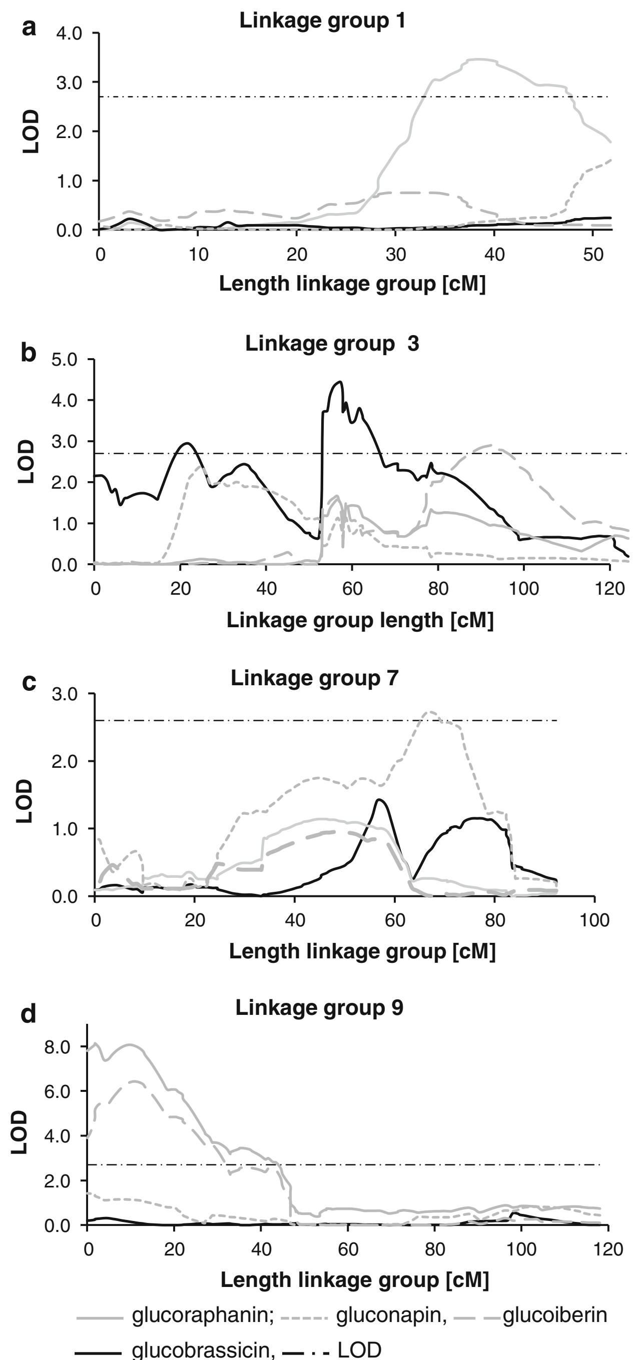


Fig. 2 QTL for the initial glucosinolate concentrations (c_0 values) of glucobrassicin, glucoraphanin, glucoiberin and gluconapin in spring 2009. The *black horizontal line* represents the genome-wide LOD value, determined with the permutation test

the k_d values of two different GLs present in the same DH lines were plotted against each other. As an example, the plot of the k_d values of the aliphatic GL glucoraphanin against the k_d values of the indolic GL glucobrassicin is shown in Fig. 4, which shows a positive linear relationship. To estimate the strength of the relationship between the k_d values of all the different GLs, the Pearson correlation coefficients and significances were calculated (Table 3). For all the possible combinations of the different GLs, positive correlations were found. A strong correlation ($r > 0.5$) was observed for all the correlations, except for neoglucobrassicin ($r < 0.5$) with glucoiberin and sinigrin, but all the correlations were significant ($p < 0.05$) and most of the correlations were highly significant ($p < 0.01$). The strongest correlations ($r > 0.9$) were observed for the aliphatic GLs, gluconapin with sinigrin and gluconapin with progoitrin. Gluconapin and sinigrin are both alkenyl GLs, which differ in their side chain only by one CH_2 group. The side chain of progoitrin consists also of an alkenyl group with the only modification, compared to gluconapin, being a hydroxyl group at the second C atom. The k_d values of glucoraphanin and glucoiberin were also highly correlated ($r = 0.886$), which are both sulfinyl GLs with one CH_2 group difference in their side chains. The correlations for GLs with structurally different side chains were less strong, for example the aliphatic GL glucoraphanin versus the indolic GL glucobrassicin ($r = 0.771$). The linear correlation of the k_d values of different GLs in the same DH lines indicates that similar factors influence the thermal degradation of structurally

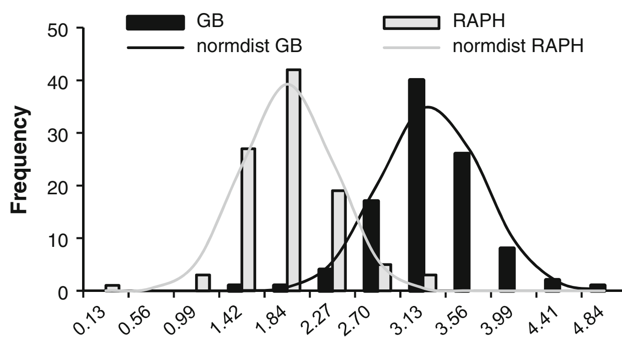


Fig. 3 Histogram of thermal degradation rate constants (k_d values) of glucoraphanin (RAPH) and glucobrassicin (GB) in the DH population in spring 2009. *Bars* represent the observed frequencies and the *lines* represent the normal distribution curve

different GLs. The influencing factors for the degradation of aliphatic GLs may differ from the influencing factors for the degradation of indolic GLs, since the correlation between k_d values of aliphatic and indolic GLs is less strong.

Quantitative trait loci analysis

To test the hypothesis if the variation in GL degradation could be explained genetically, QTL mapping was performed for GL thermal degradation using the degradation rate constants (k_d values) obtained from modeling the measured GL concentrations after several heating times using the first-order equation (Eq. 1). Two significant QTL could be identified for GL degradation (Fig. 5). The major QTL was identified on linkage group 9, which explains 13.6 % of phenotypic variation for glucobrassicin (LOD = 3.7) and 12.2 % of the phenotypic variation for glucoraphanin (LOD = 3.5). Furthermore, for the degradation of glucobrassicin, a second QTL (LOD = 3.24) was identified on linkage group 7, explaining another 11.4 % of the phenotypic variation. QTL mapping for the GLs, which were present in less than 100 DH lines, did not result in any significant results, probably due to the lower number of data. The co-localization of one QTL for the degradation of glucobrassicin and glucoraphanin suggests that there are common genetic factors involved in the degradation of the two GLs. No epistatic effects were found for the two QTL identified for the degradation of glucobrassicin using regression analysis ($p > 0.05$). As shown in Table 2, the GL degradation rate constants (k_d values) are independent of the initial GL concentration. This result was confirmed by QTL mapping of the initial GL concentrations of glucoraphanin and glucobrassicin, which did not co-localize with the QTL for GL degradation (Fig. 2).

Influence of the season on the glucosinolate thermal degradation

To test if the GL degradation was reproducible, ten DH lines selected to cover the observed range of GL degradation rate constants for glucobrassicin and glucoraphanin in spring 2009 were grown in 2011 in the same season and treated in the same way as in spring 2009. The obtained first-order degradation rate constants (k_d values) were compared (Fig. 6). The k_d values for glucoraphanin were between 23 % lower and 120 % higher in spring 2011 compared to spring 2009 (Fig. 6a) and the k_d values for glucobrassicin were between 22 % lower and 72 % higher in spring 2011 compared to spring 2009. The observed range of k_d values was smaller in spring 2011 compared to spring 2009 for glucobrassicin and glucoraphanin. Furthermore, Fig. 6 illustrates that in most of the DH lines a change in the k_d value in 2011 compared to 2009 for glucobrassicin was associated with a change in the same direction in the k_d value for glucoraphanin. For that reason,

Table 2 Variation of the glucosinolate thermal degradation (k_d values) for all the GLs identified in the DH population and the correlation with the initial glucosinolate concentrations (c_0 values) in spring 2009

Glucosinolate (present in nr. of DH lines)	Mean k_d value k_d *10^{-2} [min^{-1}]	Range k_d values k_d *10^{-2} [min^{-1}]	Max. difference (-fold)	Correlation k_d–c_0 values[ns]
Glucoiberin[a] (65)	1.26	0.66–2.04	3.1	0.038
Progoitrin[a] (45)	1.44	0.63–2.46	3.9	−0.174
Sinigrin[a] (40)	1.65	1.00–2.30	2.3	−0.191
Glucoraphanin[a] (100)	1.85	0.34–3.00	8.7	−0.044
Gluconapin[a] (60)	1.91	0.80–2.74	3.4	−0.117
Glucobrassicin[b] (100)	3.21	1.55–4.63	3.0	−0.027
Neoglucobrassicin[b] (91)	2.52	1.64–4.21	2.9	0.193

[a] Classified as aliphatic GL according to the structure of the side chain

[b] Classified as indolic GL according to the structure of the side chain

[ns] None of the correlations was significant ($p > 0.05$)

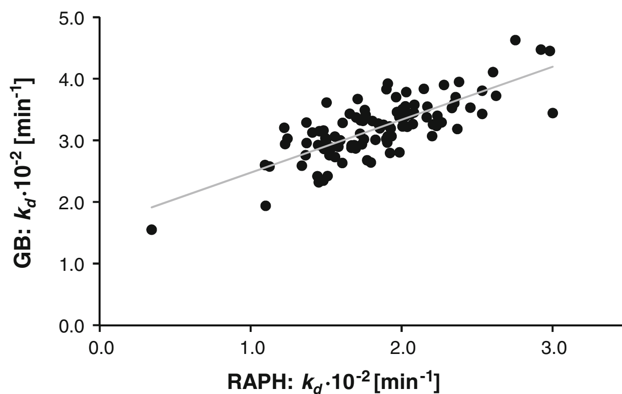


Fig. 4 Plot of the degradation rate constants (k_d values) of glucoraphanin (RAPH) against the k_d values of glucobrassicin (GB) in the DH population in spring 2009

the Pearson correlation coefficients for the k_d values of different GLs in spring 2011 were calculated (Table 4). The k_d values of the different GLs are all strongly correlated ($r > 0.5$), although only some correlations are significant. A strong correlation of the degradation of different GLs in the same DH lines was also found in spring 2009. However, the k_d values of the same GLs in spring 2009 and spring 2011 are not correlated ($r < 0.5$, not significant, Table 5). The correlation of the k_d values of different GLs within each season indicates again that there are common factors in the DH lines influencing the GL thermal degradations which are affected by the environment. The initial GLs concentrations were found to be independent of the k_d values in spring 2011 for most of the GLs (correlations not significant), similar to those shown for the DH population in spring 2009. In contrast to the k_d values, the initial GL concentrations (c_0 values) of the same GLs were significantly correlated between the seasons, except for glucobrassicin and neoglucobrassicin, which were present at low levels compared to the aliphatic GLs.

Discussion

A publicly available DH population, obtained by crossing a broccoli genotype with a Chinese kale genotype (Bohuon et al. 1996), was used to study a novel agronomic trait, GL degradation rate during food processing. A mechanistic approach was applied by studying the GL degradation solely induced by heat without degradation caused by the enzyme myrosinase. Previous research has shown that enzymatic degradation during processing of *Brassica* vegetables is far less important for GL loss than thermal degradation. Experimental data on GL thermal degradation have been modeled to obtain first-order rate constants (k_d values) to compare the degradation speed of the GLs in different DH lines. A rapid method for quantitatively determining the rate constants was used (Hennig et al. 2012b). Threefold difference in GL degradation rate constants throughout the DH population was observed for most of the GLs, except for glucoraphanin which differed by ninefold. GL degradation in the parental lines was investigated in autumn 2010 and spring 2011 (Hennig et al. 2012b). The degradation rates of structurally same GLs did not differ significantly between the parental lines, a Chinese kale and a broccoli, within each season; however, a large variation for GLs degradation rate constants in the DH progeny was observed, a phenomenon called transgressive segregation, which occurs especially if multiple genetic loci control the trait and when both parents have alleles with opposite effects at different loci (Mao et al. 2011).

Previously, thermal degradation of the GLs gluconapin, glucobrassicin and 4-methoxyglucobrassicin was studied in five different Brassica vegetables by Dekker et al. (2009). The highest difference in GL degradation rates, 21-fold, was observed for gluconapin between broccoli and Brussels sprouts, whereas a sixfold difference was found for the degradation rates of glucobrassicin and fourfold difference

Table 3 Correlation coefficients of the k_d values for glucosinolate thermal degradation of the DH population in spring 2009 for the different glucosinolates, when they were present in the same DH lines

k_d 2009	IB	PRO	SIN	RAPH	NAP	GB	NEO
IB	1	0.577*	0.586**	0.886**	0.514*	0.658**	0.410*
PRO	0.577*	1	0.871**	0.799**	0.928**	0.604**	0.557**
SIN	0.586**	0.871**	1	0.734**	0.940**	0.677**	0.489*
RAPH	0.886**	0.799**	0.734**	1	0.832**	0.771**	0.543**
NAP	0.514*	0.928**	0.940**	0.832**	1	0.750**	0.530**
GB	0.658**	0.604**	0.677**	0.771**	0.750**	1	0.584**
NEO	0.410**	0.557**	0.489*	0.543**	0.530**	0.584**	1

Aliphatic GLs: *IB* glucoiberin, *PRO* progoitrin, *SIN* sinigrin, *RAPH* glucoraphanin, *NAP* gluconapin; indolic GLs: *GB* glucobrassicin, *NEO* neoglucobrassicin

** Correlation is significant at the level 0.01 (2-tailed, corrected for multiple testing: $\alpha < 0.00048$), *Correlation is significant at the level 0.05 (2-tailed, corrected for multiple testing: $\alpha < 0.00238$)

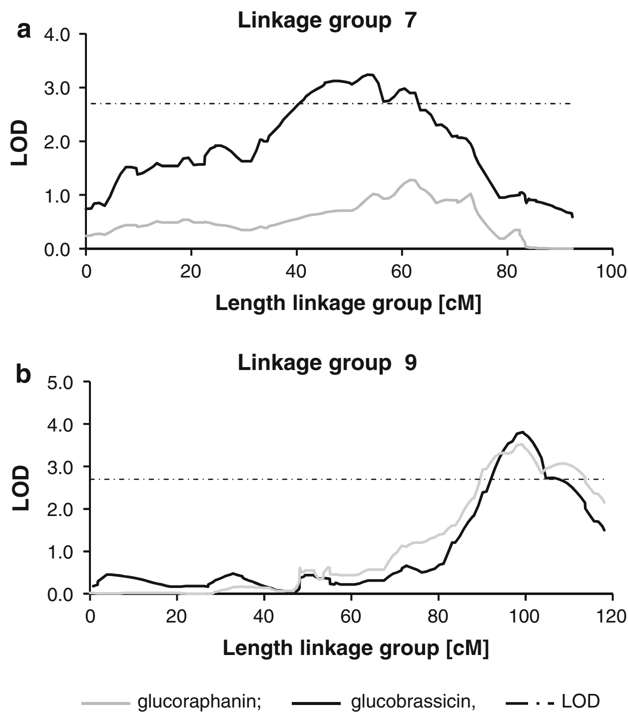


Fig. 5 QTL for glucosinolate thermal degradation of glucobrassicin and glucoraphanin in spring 2009. The *black horizontal line* represents the genome-wide LOD value, determined with the permutation test

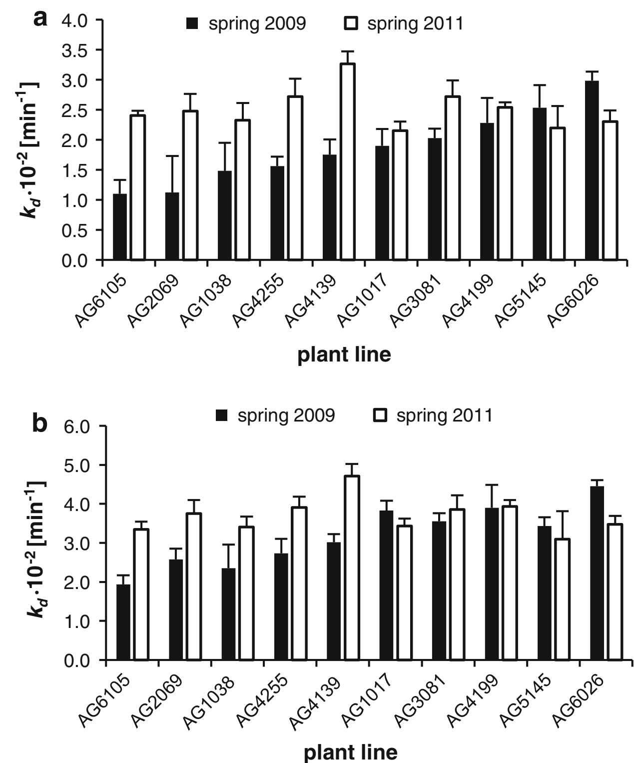


Fig. 6 Comparison of the rate constants (k_d values) in a subset of the DH population grown in spring 2009 and in spring 2011 for glucoraphanin (**a**) and glucobrassicin (**b**). The *bars* represent obtained k_d values from a first-order model and the *error bars* represent the standard deviations

for 4-methoxyglucobrassicin between red cabbage and Brussels sprouts. Dekker et al. (2009) suggested differences in the reaction environment caused by the different plant matrices of the vegetables as a reason for the differences in GL degradation. The term "plant matrix" refers here to the metabolite composition which is the biochemical basis of a vegetable, since fully ground tissue was used to study GL thermal degradation and hence the cell and tissue structure did not influence it. In our study, the differences in GL degradation rates throughout the DH population were smaller than in the study of Dekker et al. (2009), who investigated very different edible parts of each studied vegetable, the inflorescences of broccoli, the axillary buds of Brussels sprouts and the leaves of the cabbages and Pak Choi at commercial maturity, bought in a supermarket. The vegetables were likely produced in different geographical regions and had different transport and storage histories. So the observed differences in that case were the combined effect of growing conditions, genetic variation, developmental variation and plant age. The DH lines of the DH population in this study were grown at the same conditions and the same organs were investigated, which is likely the reason for smaller differences in the plant matrix and hence a smaller differences in the GL degradation rates. Additionally, the DH population reflects only the genetic variation of two parents, which is only part of the variation within the species. Identified factors in the plant matrix that influence GL degradation rates are pH and water content (Hanschen et al. 2012b; Oliviero et al. 2012).

Table 4 Correlation coefficients for glucosinolate thermal degradation (k_d values) of a subset of the DH population (10 lines) in 2011, when present in the same DH lines

k_d 2011	IB	PRO	SIN	RAPH	NAP	GB	NEO
IB	1	0.963	0.862	0.929*	0.894	0.874	0.973*
PRO	0.963	1	0.881	0.976	0.963	0.961	0.920
SIN	0.862	0.881	1	0.871	0.919	0.809	0.785
RAPH	0.929*	0.976	0.871	1	0.867	0.944*	0.898*
NAP	0.894	0.963	0.919	0.867	1	0.878	0.971
GB	0.874	0.961	0.809	0.944*	0.878	1	0.835
NEO	0.973*	0.920	0.785	0.898*	0.791	0.835	1

Aliphatic GLs: *IB* glucoiberin, *PRO* progoitrin, *SIN* sinigrin, *RAPH* glucoraphanin, *NAP* gluconapin, indolic GLs: *GB* glucobrassicin, *NEO* neoglucobrassicin

* Correlation is significant at the level 0.05 (2-tailed, corrected for multiple testing: $\alpha < 0.00238$)

In our study we could identify two QTL for the degradation of glucobrassicin, explaining in total 25 % of the explained variation. One QTL was identified for the

Table 5 Correlation coefficients for glucosinolate thermal degradation (k_d values) and the initial glucosinolate concentrations (c_0 values) of a subset of the DH population (10 lines) in 2011 and correlation with values determined in the same DH lines in 2009, when present in the same DH lines

Glucosinolate	Correlation k_d–c_0 values 2011	Correlation k_d 2009–2011	Correlation c_0 2009–2011
Glucoiberin	0.160	−0.257	0.926**
Progoitrin	0.963**	0.063	0.998**
Sinigrin	0.599	0.281	0.992**
Glucoraphanin	0.356	−0.217	0.944**
Gluconapin	−0.261	0.309	0.997**
Glucobrassicin	−0.195	0.005	0.541
Neoglucobrassicin	0.386	0.353	−0.91

** Correlation is significant at the level 0.01 (2-tailed); * Correlation is significant at the level 0.05 (2-tailed)

degradation of gluoraphanin, explaining 12 % of the phenotypic variation, which co-localized with one of the QTL identified for glucobrassicin degradation. With these results the hypothesis that there is a (partly) genetic regulation of GL degradation, can be accepted. The genetic regulation of GL thermal degradation is most likely indirectly via factors in the plant matrix influencing the stability of GLs during heat treatment. The two QTL identified for the degradation of glucobrassicin and the transgressive segregation show that GL degradation is a complex trait with several loci involved. Furthermore, the GL degradation appears to be strongly affected by the season and year. In the parental lines, thermal degradation was between 20 and 80 % higher in spring 2012 compared to autumn 2011 depending on the type of GL (Hennig et al. 2012b). In contrast to the GL degradation in the parental lines, the degradation in a subset of the DH population (10 DH lines) was in some DH lines increased and in some others decreased between the two seasons, which indicated that the factors that formed the plant matrix were differently affected by the season in different DH lines. The correlation of the degradation of different GLs within both seasons suggests that the same factors in one DH line influence the degradation rate of different GLs. These factors, which may be specific metabolites, are assumed to be not only affected by the genotype of the DH line, but also by the environment, since the GL degradation of the same GL was not correlated between the two years.

The presented correlation data demonstrate that the speed of GL degradation is independent of the initial GL concentration in the DH population. Furthermore, the identified QTL for GL degradation do not co-localize with the identified QTL for the initial GL concentration. Several studies have been conducted to elucidate the genes involved in GL biosynthesis, such as the AOP (2-oxoglutarate-dependent dioxygenase), MAM (methylthioalkylmalate synthase) and MYB family (Kliebenstein et al. 2001a, b; Sonderby et al. 2010). Lou et al. (2008) predicted the AOP genes on two homeologous loci, one on linkage group 3 and another on linkage group 9 in *Brassica rapa*. The QTL for the initial GL concentration in this *B. oleracea* population mapped on linkage group 3 and at the top of linkage group 9; the genes underlying these QTL are possibly AOP due to the synteny between the *B. oleracea* and *B. rapa* genome.

The precise factors underlying the differences in GL degradation between the different DH lines remain unknown. If oxygen played a role in GL thermal degradation, antioxidants are possible substances for preventing GL degradation. Possibly, a combination of several factors determines the GL degradation speed in a DH line. The identification of GL thermal degradation products in broccoli sprouts showed that the corresponding nitriles were the dominant breakdown products and the corresponding isothiocyanates were formed in minor quantities (Hanschen et al. 2012a). Since the thermal breakdown products are identical to the enzymatic breakdown products formed by myrosinase (only the profile is different), it can be speculated that during heating the glucose is hydrolyzed from the GL leaving the same unstable intermediate as in the enzymatic breakdown, which rearranges into nitriles and isothiocyanates. However, the breakdown products formed do not provide information about the factors influencing GL degradation. The identified QTL regions are too large to scan for orthologs of genes with an assigned function in *Arabidopsis*, because there is no indication of which type of genes can cause differences in GL degradation. Fine mapping of the DH population is an option to narrow down the number of genes. Further analysis of the metabolic composition of the DH lines will shed light on the factors retarding or increasing GL degradation.

QTL studies for food processing traits have not been performed frequently; only two examples were found in literature: one on the color retention during canning of black beans (Wright and Kelly 2011) and the other on the pasting properties of barley (Wang et al. 2010). Both authors identified multiple QTL on several chromosomes which were affected by environmental and growth conditions. These studies show, similar to the presented study on GL degradation, that food-processing traits are complex and the genes underlying these traits are not known or only partly known. QTL studies could not only facilitate marker-assisted breeding to select for varieties that perform better during food processing, but also represent the first steps toward identification of the genes responsible for these QTL. Identifying these genomic regions is the first step toward understanding the mechanism of glucosinolate degradation and identification of factors that influence this degradation.

In conclusion, this study shows that QTL mapping for GL degradation rate constants during food processing is possible and provides new opportunities to develop vegetables with increased retention of their health-promoting properties during industrial processing or home cooking. Breeding for vegetables with a high retention of GLs during processing can complement the development of vegetables with high initial GL content to obtain a high concentration of health promoting compounds at the stage of consumption.

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