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Rapid Estimation of Glucosinolate Thermal Degradation Rate Constants in Leaves of Chinese Kale and Broccoli (*Brassica oleracea*) in Two Seasons

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ABSTRACT: Kinetic modeling was used as a tool to quantitatively estimate glucosinolate thermal degradation rate constants. Literature shows that thermal degradation rates differ in different vegetables. Well-characterized plant material, leaves of broccoli and Chinese kale plants grown in two seasons, was used in the study. It was shown that a first-order reaction is appropriate to model glucosinolate degradation independent from the season. No difference in degradation rate constants of structurally identical glucosinolates was found between broccoli and Chinese kale leaves when grown in the same season. However, glucosinolate degradation rate constants were highly affected by the season (20−80% increase in spring compared to autumn). These results suggest that differences in glucosinolate degradation rate constants can be due to variation in environmental as well as genetic factors. Furthermore, a methodology to estimate rate constants rapidly is provided to enable the analysis of high sample numbers for future studies.

KEYWORDS: *Brassicaceae, glucosinolates, glucosinolate breakdown, kinetic modeling, model discrimination, Brassica oleracea var. alboglabra, Brassica oleracea var. italica, seasonal effects, thermal degradation*

INTRODUCTION

Kinetic modeling is a mathematical tool with which chemical changes in foods can be described in a quantitative way.[1] Modeling has been used to study the effect of food processing on the concentration of health-promoting compounds, because the awareness on the relation of food and health has increased over the last few decades.[2] One group of bioactive compounds comprises the glucosinolates (GLs), which occur in high concentrations in plants of the Brassicaceae family. The basic chemical structure of GLs is a β-thioglucoside-*N*-hydroxy sulfate with a sulfur-linked β-D-glucopyranose moiety and a side chain (R), which determines the class of GLs (aromatic, indolic, and aliphatic).[3] The biological activity of GLs is ascribed to their breakdown products, which are obtained by the enzymatic conversion of GLs by the endogenous plant enzyme myrosinase (β-thioglycosidase, EC 3.2.1.147).[4] Dependent upon the reaction conditions, several types of breakdown products can be formed, such as isothiocyanates, thiocyanates, epithionitriles, and nitriles, from which isothiocyanates are most biologically active.[5] Breakdown products of specific GLs contribute to the typical flavor of *Brassica* vegetables, and in particular, isothiocyanates have been associated with a lower risk of cancer.[3]

The majority of *Brassica* vegetables is consumed after domestic cooking, which leads to inactivation of myrosinase and leaves intact GLs.[6] Intact GLs can be hydrolyzed by the gut microbiota into breakdown products, but the conversion rate is less than after ingestion of vegetables with intact myrosinase.[7,8] Several mechanisms of GL losses during food processing have been described: (i) enzymatic breakdown by myrosinase upon cell lysis, (ii) leaching of GLs into the cooking water, and (iii) thermal degradation.[2] The term thermal degradation refers to the degradation solely induced by heat, without any enzymatic reactions. Next to leaching of GLs into the cooking water, thermal degradation is one of the major mechanisms leading to losses of GLs during food processing, because myrosinase is inactivated quickly in most processes.[2] Thermal degradation leads to the formation of nitriles, which are potentially toxic[9] and, thus, undesired during food processing. To quantitatively study thermal degradation in isolation, the plant material should be treated to inactivate myrosinase and heated in the absence of a separate water phase.

Thermal degradation has been studied in this way in red cabbage and broccoli sprouts, showing that the GL degradation is dependent upon the structure of the GL side chain.[10,11] The degradation kinetics were described by a first-order model.[11] A study comparing the thermal degradation of GLs for five different vegetables showed that the stability of structurally identical GLs differed in different vegetables (broccoli, Brussels sprouts, red cabbage, Chinese cabbage, and pak choi). The five studied vegetables have different matrices and, hence, provide different reaction environments for thermal degradation.[12]

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To obtain more insight into the causes of differences in thermal degradation, well-characterized plant material is required to exclude differences in degradation because of different growing locations, seasons, age of the plants, storage time, and storage conditions. All of the mentioned factors possibly influence the food matrix and, hence, the thermal degradation. It has been shown that the initial GL concentrations in vegetables are affected by the season.[13] Consequently, the question arises if the thermal degradation is affected by the season as well.

The aims of this investigation were to study the effect of the genotype and season on GL thermal degradation in well-characterized plant material and to develop a rapid method to quantitatively determine the thermal degradation rate constants. This information will provide the methodology for future experiments to study in detail the genetic and environmental effects on GL thermal degradation in a more efficient way.

Knowledge of the genetic factors influencing GL degradation will enable plant breeders to develop vegetables with low GL degradation, resulting in higher GL concentrations at the point of consumption to increase health benefits for the consumer.

MATERIALS AND METHODS

Plant Material. A rapid-cycling Chinese kale doubled haploid (DH) line, *Brassica oleracea* var. *alboglabra* (A12DHd), and a broccoli DH line, *Brassica oleracea* var. *italica* (GDDH33), are the parental lines of a DH population developed by Bohuon et al.[14] For the sake of convenience, the genotype A12DHd will be referred to as Chinese kale and the genotype GDDH33 will be referred to as broccoli. Five plants of each genotype were grown in autumn 2010 and spring 2011. Seeds were sown into soil, transplanted into 19 cm diameter pots after 2 weeks, and grown randomized for 6 weeks after transplanting in an air-conditioned greenhouse in Wageningen (The Netherlands). Fertilizer was given 2−3 times per week (electric conductivity of 2.1). In autumn 2010 (the middle of October until the middle of December), temperatures ranged from 17 to 22 °C (night/day) and a photoperiod of 16 h was applied. In spring 2011 (the end of March until the end of May), temperatures were set to 17/22 °C (night/day); however, on sunny and warm days, the temperature could rise to 30 °C during the afternoon, because greenhouses are not cooled. Artificial light was applied if the natural photoperiod was shorter than 16 h.

At 6 weeks after transplanting, all of the leaves without petioles from five plants per genotype were harvested in the morning and transported on ice to the laboratory for further sample preparation. All leaves of the five plants per genotype were mixed to prepare one homogeneous sample. To inactivate the enzyme myrosinase while maintaining a high level of GLs, a microwave treatment was performed at high power for a short time as described previously.[11,15] This allowed for the study of the thermal degradation as the sole mechanism of GL degradation. Leaves were cut into pieces of about 3 × 3 cm, and 75 g was collected 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. Samples were immediately cooled on ice; the 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 high-performance liquid chromatography (HPLC) grade and bought from Biosolve (Valkenswaard, The Netherlands). The (diethylamino)ethyl (DEAE) Sephadex A-25 and sinigrin (prop-2-enylglucosinolate) were purchased from Sigma-Aldrich (Zwijndrecht, The Netherlands). The internal standard glucotropaeolin (benyzlglucosinolate) was purchased from the Laboratory of Biochemistry, Plant Breeding and Acclimatization Institute (Radzikow, Błonie, Poland). Sulfatase from *Helix pomatia* (Sigma-Aldrich, Zwijndrecht, The Netherlands) was used for the experiments.

Thermal Degradation. Dried plant powder was weighed into 2 mL plastic tubes with screw caps and reconstituted to the water content present in the plant before microwave treatment and drying. Varying amounts of dried plant powder were weighed into tubes, and 180 μL of water was added to each tube and mixed. Tubes with reconstituted samples were heated in a heating block at 100 °C for 0, 15, 30, 45, 60, 75, 90, 105, and 120 min. All heat treatments 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. Time recording started when the tubes were placed into the heating block. After heating, samples were cooled on ice and stored at −20 °C until GL analysis.

GL Analysis. Samples were prepared by heating for nine different time points (in duplicate) per genotype and season. Each sample was prepared once for GL analysis. The GLs were analyzed as desulfo-GLs according to Hennig et al.,[16] which is based on ISO 9167-1:1992. The reconstituted and heated samples (about 200 mg) were extracted with 1 mL of hot methanol (70%, 75 °C) for 10 min and centrifuged for 5 min at room temperature at 16000*g*. Glucotropeaolin was added as an internal standard (3 mM, 25 μL) during the first extraction. The pellet was re-extracted in the same way, and the combined supernatants were used for desulfation. DEAE Sephadex A-25 columns were built in 96-well microtiter-filter plates (Millipore, Amsterdam, The Netherlands). To pack the columns, to remove rinsing liquids, to load, and to elute the samples, the microtiter plates were centrifuged for 5 min at 900*g*. The GL extracts were loaded in two aliquots of 1 mL on the ion-exchange columns and subsequently rinsed twice with 500 μL of sodium acetate buffer (20 mM, pH 4.0). For desulfation of GLs, 100 μL of freshly purified sulfatase solution was added to the ion-exchange columns and incubated for 16 h at 25 °C. Desulfo-GLs were eluted after incubation in microtiter plates by adding 100 μL of water twice.

Desulfo-GLs were analyzed by HPLC as described by Verkerk et al.[17] Identification of GLs was based on retention times compared to standard GLs (sinigrin and glucotropaeolin), GLs present in the European Reference Material Rapeseeds (Sigma-Aldrich, Zwijndrecht, The Netherlands), GLs present in reference vegetables, and UV spectra of GLs.[18] GLs were quantitated against the internal standard glucotropaeolin using relative response factors as given in ISO 9167-1:1992 and recalculated to glucotropeaolin. The initial GL concentrations prior to heating were determined after the myrosinase inactivation step.

Modeling. Modeling of GL degradation has been performed using all of the data for each genotype and season separately. The molecular mechanism of GL thermal degradation is not known. The general rate law for a single reactant at a concentration c is described in eq 1, with the rate of the reaction r, the concentration c, the reaction rate constant k, and the order of the reaction n.[19] The order of a reaction describes the dependence of the reaction rate upon the reactant concentration. In contrast to elementary reactions, n can be fractional and does not necessarily correspond to the stoichiometry of the reaction.[19]

$$r = -\frac{dc}{dt} = kc^n \quad (1)$$

The integral with respect to time of the general rate law allows us to follow the GL degradation over time (eqs 2 and 3),[19] with c being the GL concentration at a certain time point, c_0 being the GL concentration at time zero (prior to heating), k_d being the degradation rate constant, t being the time, and n being the order of the reaction.

$$c = [c_0^{(1-n)} + (n-1)k_d t]^{1/1-n} \quad \text{for } n \neq 1 \quad (2)$$

$$c = c_0 e^{-k_d t} \quad \text{for } n = 1 \quad (3)$$

Kinetic parameters (k_d and c_0 for the first-order reaction and k_d, c_0, and n for the nth-order reaction) were obtained by fitting the model through the individual data points by minimizing the sum of squared

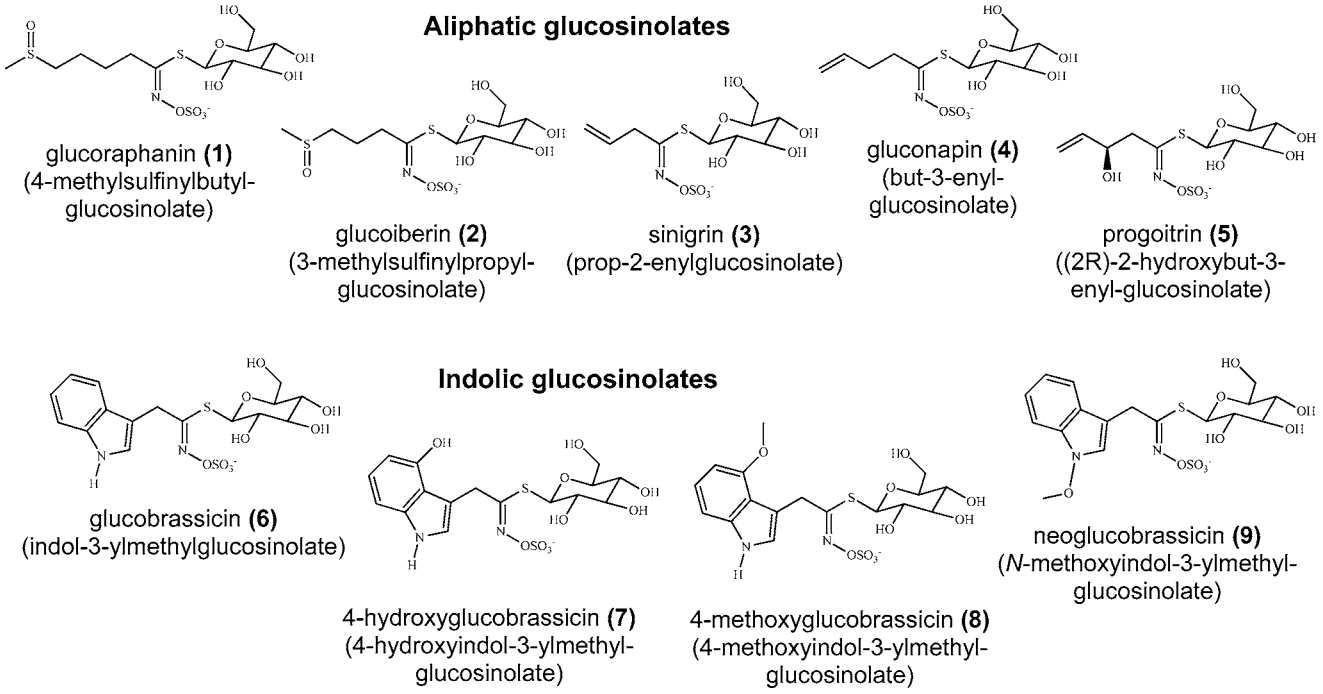

Figure 1. Chemical structures and trivial names of the GLs identified in leaves of a broccoli and a Chinese kale genotype used in this study.

Table 1. Total and Individual GL Concentrations in Broccoli and Chinese Kale in Two Different Seasons[a]

	broccoli		Chinese kale	
	average ± standard deviation (μmol/100 g of FW)		average ± standard deviation (μmol/100 g of FW)	
GL	autumn 2010	spring 2011	autumn 2010	spring 2011
glucoiberin		13.13 ± 0.70 a	2.67 ± 0.03 b	2.99 ± 0.05 b
progoitrin			1.19 ± 0.00 a	0.35 ± 0.05 b
sinigrin			11.24 ± 0.19 a	7.64 ± 0.10 b
glucoraphanin	15.89 ± 0.51 a	22.22 ± 1.12 b	4.07 ± 0.05 c	6.66 ± 0.54 c
gluconapin			27.90 ± 0.64 a	23.63 ± 0.08 b
4-hydroxy-glucobrassiscin	0.46 ± 0.01 a	1.97 ± 0.08 b	0.32 ± 0.07 a	0.42 ± 0.01 a
glucobrassicin	22.20 ± 1.05 a	10.50 ± 0.37 b	7.07 ± 0.09 c	4.63 ± 0.08 d
4-methoxy-glucobrassicin	1.65 ± 0.03 a	2.94 ± 0.05 b	0.72 ± 0.01 c	1.16 ± 0.00 d
neoglucobrassicin	4.44 ± 0.04 a	2.57 ± 0.19 b	0.53 ± 0.02 c	0.39 ± 0.03 c
total GLs	44.65 ± 1.61 a	53.33 ± 2.50 b	55.72 ± 1.05 b	47.88 ± 0.58 a

[a]Different letters indicate significant differences between the different genotypes and seasons for each GL ($p < 0.05$; Tukey's HSD test and t test if the GL was present in Chinese kale only).

residuals[1] using the solver function in Microsoft Excel. The macro Solver Aid was used to obtain the standard deviation of the parameters and the linear correlation coefficients of the parameters.[20,21] The corrected Akaike information criterion (AIC_c) was used to discriminate between the first- and nth-order models (eq 4),[19] with m being the number of data points, SS_r being the sum of squared residuals, and p being the number of parameters. For model discrimination, only the differences between the AIC_c for the different models is relevant (eq 5).[19] The probability P_{AIC} to estimate which model is more likely was calculated using eq 6. If P_{AIC} is smaller than 0.5, the first-order model is more likely, whereas if P_{AIC} is greater than 0.5, the nth-order model is more likely.[19]

$$AIC_c = m \ln\left(\frac{SS_r}{m}\right) + 2(p+1) + 2(p+1)\left(\frac{p+2}{m-p}\right) \quad (4)$$

$$\Delta AIC_c = AIC_{c,nth} - AIC_{c,first} \quad (5)$$

$$P_{AIC} = \frac{e^{(-0.5\Delta_{AIC})}}{1 + e^{(-0.5\Delta_{AIC})}} \quad (6)$$

Statistics. Means of the initial GL concentration were compared using one-way analysis of variance (significance level $p < 0.05$), followed by a Tukey's honestly significant different (HSD) test to test for differences between individual groups using the software IBM SPSS statistics 19. To test for significances of the k_d values between two genotypes and the two seasons, t tests have been performed. The standard deviations of the k_d values were obtained by the macro SolverAid, and the degrees of freedom were calculated from the number of data points reduced by the number of parameters. Furthermore, a paired t test was applied to test for significances of the k_d values of all GLs between both seasons (Microsoft Excel 2010).

RESULTS AND DISCUSSION

Variation of the Initial GL Concentration in Different Seasons. Nine different GLs were identified in leaves of Chinese kale and broccoli used in this study (Figure 1). The broccoli leaves contained glucoraphanin (4-methylsulfinylbutylglucosinolate, **1**), glucobrassicin (indol-3-ylmethylglucosinolate, **6**), 4-hydroxy-glucobrassicin (4-hydroxyindol-3-ylmethyl-

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Table 2. Estimated Parameters by Fitting Different Models through the Measured GL Concentrations after Various Heating Times (0−120 min)[a]

		*n*th-order model				first-order model			
genotype	season	n	SD (n)	k_d ($\times 10^{-2}$, μmol$^{(n-1)}$ min^{-1})	SD (k_d) (%)	k_d ($\times 10^{-2}$, min^{-1})	SD (k_d) (%)	Δ_{AIC}	P_{AIC}
				Glucoraphanin					
B	autumn 2010	1.70	0.24	8.4	59.6	1.4	6.7	−7.2	0.97[b]
CK	autumn 2010	1.48	0.18	9.5	67.6	1.5	5.0	−6.1	0.96[b]
B	spring 2011	1.09	0.11	3.2	24.4	2.6	1.4	2.2	0.25[c]
CK	spring 2011	1.06	0.11	2.9	37.7	2.4	3.9	2.8	0.20[c]
				Glucobrassicin					
B	autumn 2010	1.18	0.07	4.5	16.6	3.0	3.5	−4.1	0.89[c]
CK	autumn 2010	0.99	0.07	3.0	23.0	3.1	2.8	2.9	0.19[b]
B	spring 2011	0.87	0.06	2.5	19.5	3.7	3.1	−1.3	0.66[c]
CK	spring 2011	0.88	0.08	2.4	32.3	3.8	3.8	1.1	0.36[b]

[a]B, broccoli; CK, Chinese kale; k_d, rate constant; n, reaction order; SD, standard deviation; AIC_c, corrected Akaike information criterion; Δ_{AIC}, $AIC_{nth} - AIC_{first}$; and P_{AIC}, probability of the AIC ($P_{AIC} < 0.5$, the first-order model is more likely; $P_{AIC} > 0.5$, the nth-order model is more likely). [b]nth-order model is more likely. [c]First-order model is more likely.

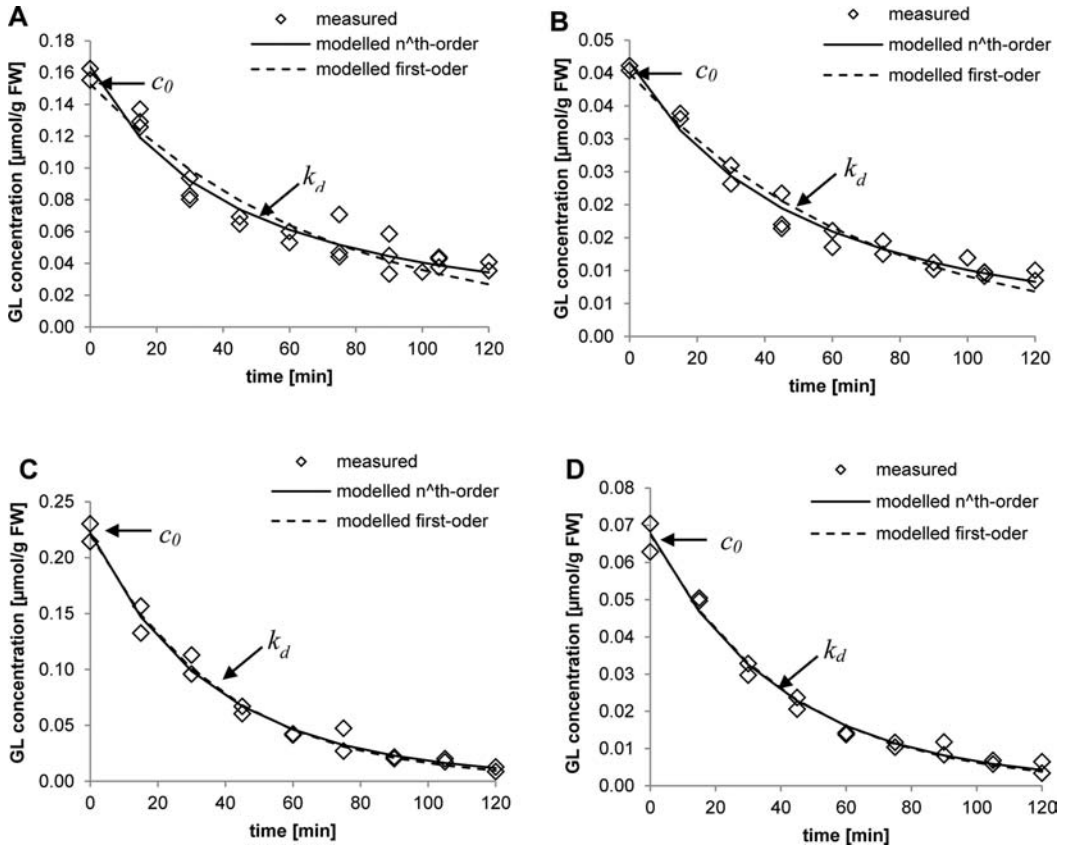

Figure 2. First- and nth-order fits through the measured data for glucoraphanin in (A) broccoli autumn 2010, (B) Chinese kale autumn 2010, (C) broccoli spring 2011, and (D) Chinese kale spring 2011.

glucosinolate, **7**), 4-methoxy-glucobrassicin (4-methoxyindol-3-ylmethylglucosinolate, **8**), and neoglucobrassicin (*N*-methoxyindol-3-ylmethylglucosinolate, **9**). Glucoiberin (3-methylsulfinylpropyl-glucosinolate, **2**) was detected in spring 2011 only. In Chinese kale, leaves contained the same GLs and additionally sinigrin (prop-2-enylglucosinolate, **3**), gluconapin (but-3-enylglucosinolate, **4**), and progoitrin [(2*R*)-2-hydroxybut-3-enylglucosinolate, **5**] (concentrations shown in Table 1). The total GL concentration, calculated as the sum of the individual GLs, differs significantly between the broccoli and Chinese kale in the same season and also between the two seasons for each genotype. In broccoli, the total GL concentration was increased in spring 2011 compared to autumn 2010, which is in accordance with results from Charron et al.,[22] who found increased GL concentrations in the spring season compared to the autumn season. The total GL concentration decreased in Chinese kale in spring 2011 compared to autumn 2010 that may be explained by the different developmental stages in the two seasons. The Chinese kale plants were already bolting at the time of harvest in spring 2011 and not in autumn 2010, and GL concentrations are changing during plant development.[23]

Model Discrimination for GL Degradation. Modeling of GL degradation provides a good tool to quantitate the overall degradation progression over time. The obtained parameters

facilitate the comparison of data to study genetic and environmental effects of GL degradation. Parameters can be used by the food industry to predict GL concentrations at other heating time points. The aim of the study is to compare thermal degradation of structurally identical GLs in leaves of a broccoli and a Chinese kale genotype grown in autumn and spring seasons. Glucoraphanin (**1**) and glucobrassicin (**6**), the most abundant GLs in both genotypes, were used to determine the best fitting model. For model discrimination, experimental GL concentrations were fitted to the *n*th- and first-order models (eqs 2 and 3). Table 2 presents the parameters of most interest for both models and both GLs. The reaction order *n* was estimated close to one for glucoraphanin in broccoli and Chinese kale in spring 2011 and for glucobrassicin in Chinese kale in both seasons, which is in accordance with previous results.[11,12] However, the order deviated from one to the other GL−season combinations. The AIC_c confirms these results (Table 2). The graphs in Figure 2 demonstrate the difference between the models for glucoraphanin.

Comparing the resulting degradation rate constants k_d of the first- and *n*th-order models (Table 2) shows that the k_d values differ considerably between the two models. This is as expected. When the order differs the concentration term in the rate eq 1 is changing, and therefore, very different rate constants are estimated at the same reaction rates. To compare the k_d values among different plants and seasons, one common order for all of the plants thus has to be chosen. The introduction of one more parameter to a model increases the uncertainty of the parameters, and they could become less meaningful; hence, the uncertainty is an important factor to discuss in model discrimination. The standard deviations of the k_d values were much higher in the models with the *n*th-order equation, ranging from 17 to 60% of the estimated k_d values. Contrarily, the standard deviations of the k_d values estimated with a first-order reaction were all below 7%. Hence, the k_d values estimated with the first-order equation were more precise and more meaningful than the k_d values estimated with the *n*th-order equation. This fact shows that the *n*th-order model fitted better, but the parameters were less meaningful for the purpose of this study.

Seasonal and Genetic Effects on GL Thermal Degradation. The k_d values of all GLs at 100 °C are presented in Figure 3. The GLs 4-hydroxy-glucobrassicin (**7**) and progoitrin (**5**) in spring 2011 in Chinese kale could not be modeled, because the initial concentrations were too low to quantitate their concentration after heating. Figure 3 clearly shows higher degradation rate constants for the indolic GLs (**6**−**9**) compared to the aliphatic GLs (**1**−**5**), which is in accordance with previous results.[11,12] The order of k_d values of all GLs was the same in the two genotypes Chinese kale and broccoli grown in the two seasons (from lowest to highest k_d value): glucoiberin < progoitrin < sinigrin < glucoraphanin < gluconapin < neoglucobrassicin < glucobrassicin < 4-methoxy-glucobrassicin. Experiments with five different *Brassica* vegetables showed the same order of degradation at 100 °C.[12]

Additionally, our experiments point out that the GL degradation is significantly higher in spring 2011 compared to autumn 2010 for all GLs in both genotypes ($p < 0.01$; paired *t* test). In spring 2011, the k_d values for all GLs analyzed were for Chinese kale between 20 and 80% higher and for broccoli between 16 and 80% higher than in autumn 2010. The difference between the seasons was lower for indolic GLs than for aliphatic GLs in both genotypes (Figure 3). The observed differences in k_d for the same GLs in the two genotypes are

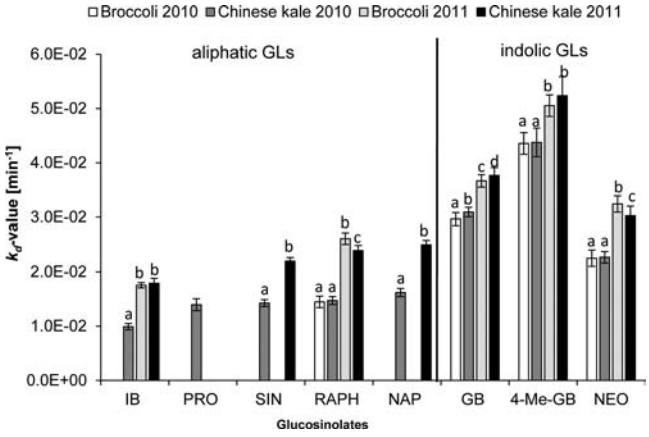

Figure 3. Rate constants (k_d values) of GL thermal degradation at 100 °C in broccoli and Chinese kale in autumn 2010 and spring 2011. The bars show the modeled k_d values, and error bars represent the standard deviations of the parameters. IB, glucoiberin; PRO, progoitrin; SIN, sinigrin; RAPH, glucoraphanin; NAP, gluconapin; GB, glucobrassicin; 4-Me-GB, 4-methoxy-glucobrassicin; and NEO, neoglucobrassicin. Missing bars indicate the absence of the GL or the levels were too low for modeling. The letters indicate significant differences ($p < 0.05$).

generally small, and only a few are significantly different. This result is in contrast to previous results that showed that the degradation of the same GLs differed in five different *Brassica* vegetables.[12] Dekker et al.[12] suggested differences in the vegetable matrix and, hence, differences in the reaction environment for the thermal degradation as reason for the differences. Other authors showed that the pH influenced thermal degradation of GLs, being lower in neutral and slightly acidic medium, whereas degradation was increased in basic medium.[10] Water, as a mediator of chemical reactions, influences the degradation of GLs as well. It has been shown that GL thermal degradation is increasing with a decreasing water content, except when water contents are very low (13%).[24] In the study by Dekker et al.,[12] the GL degradation was determined in the edible parts of five different vegetables, red cabbage, Brussels sprouts, broccoli, Chinese cabbage, and pak choi, at their commercial maturity. Hence, different plant organs were studied at different developmental stages of the plants. Furthermore, the vegetables were bought in a supermarket; thus, the growing and storage conditions were not known. In this study, we can separate the genetic and environmental effects on degradation, because we used leaves of broccoli and Chinese kale grown under two defined conditions (spring 2010 and autumn 2011), which were analyzed immediately after harvest, without storage. This resulted in the same degradation speed for several GLs in broccoli leaves and Chinese kale leaves. Our data also clearly showed that the season has a big impact on both GL concentration and GL degradation, suggesting that the differences between different vegetables in the study by Dekker et al.[12] can be caused by seasonal, environmental, and genetic effects. Studying the GL degradation in a segregating plant population, such as the AGDH DH population developed from a F1 of a cross between the broccoli and the Chinese kale used in this study,[14] could answer the question regarding the effects of environment and genetics on GL degradation. With knowledge of genetic factors on GL degradation, vegetables with a higher retention of GLs during food processing can be bred. With knowledge of the environmental influence on GL

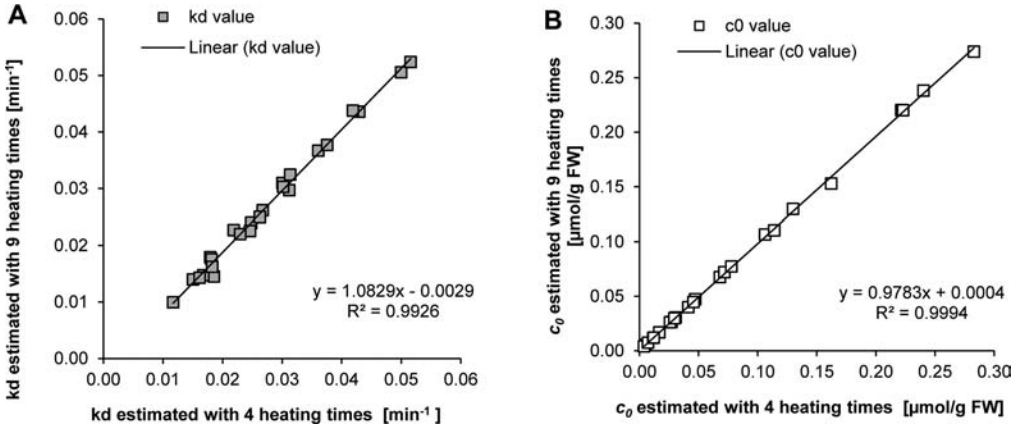

Figure 4. Plots of estimated parameters using nine heating times and four heating times for the first-order modeling. Data of all GLs present in broccoli and Chinese kale in two different seasons were modeled: (A) degradation speed (k_d values) and (B) initial GL concentration (c_0 value).

degradation, the optimal genotype−environment combination can be selected. This strategy provides a novel opportunity for the food industry to improve the level of health-promoting compounds at the stage of consumption.

Improving the Methodology To Estimate Rate Constants Rapidly. In a follow up study, we want to quantitate the genetic effect of GL thermal degradation, for which a large segregating population is needed, the progeny from the parental genotypes described in this study. These large data (>100 progeny) require high-throughput GL degradation studies; one step toward this is the use of less heating time points for GL determination. To investigate this possibility, the data set of the two investigated genotypes grown in both seasons was modeled using the first-order reaction (eq 3) in two ways: with all nine different heating times and with a subset of four heating times (0, 15, 30, and 60 min). In total, 24 data sets of GLs were modeled. The obtained kinetic parameters (k_d and c_0) estimated with nine heating times were plotted against the parameters estimated with four heating times (Figure 4). The slope amounted to 1.08 for the k_d value (Figure 4A) and to 0.98 for the c_0 value (Figure 4B), which shows that almost equal parameters are obtained by modeling with four data points (k_d value, R^2 = 0.993; c_0 value, R^2 = 0.999). The average difference between the parameters estimated with nine and four heating times amounted to 2.1% for the k_d value and to 0.8% for the c_0 value, which are lower than the average standard deviations of the estimated parameters with nine heating times (4.7% for k_d and 2.5% for c_0) and, hence, confirm the results of Figure 4. As expected, the standard deviations of the parameters estimated with four heating times were higher than after estimation with nine heating times. An average standard deviation from the 24 models of 6.8% for the k_d value and 2.8% for the c_0 after modeling with four heating time points were considered to be acceptable.

All of the data show that four heating times (0, 15, 30, and 60 min) are sufficient to estimate the k_d value, which will be used for determination of GL thermal degradation in a high number of samples in future studies to study the genetic regulation of GL composition and thermal degradation.

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