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Thermal degradation of glucosinolates in red cabbage

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Abstract

Thermal degradation of individual glucosinolates within the plant matrix was studied. Red cabbage samples were heated at different temperatures for various times. To rule out the influence of enzymatic breakdown and to focus entirely on the thermal degradation of glucosinolates, myrosinase was inactivated prior to the thermal treatments. All identified glucosinolates degradation when heated at temperatures above 100 °C. The indole glucosinolates 4-hydroxy-glucobrassicin and 4-methoxyglucobrassicin showed the highest degree of showed degradation, even at temperatures below 100 °C.

Kinetic parameters have been estimated for the degradation that could be described by first-order kinetics. At temperatures below 110 °C indole glucosinolates have a significant higher degradation rate constant as compared to aliphatic glucosinolates. The breakdown of 4-hydroxyglucobrassicin seems to consist of two parallel reaction pathways. Based on the proposed degradation kinetics and the estimated parameters, the degree of thermal degradation of all individual glucosinolates at standardized heating conditions (blanching, cooking and canning) was simulated. Glucosinolates are expected to be not very susceptible to thermal degradation during blanching conditions. Cooking will cause more thermal degradation to indole glucosinolates (38%) as compared to aliphatic glucosinolates (8%). Canning, the most severe heat treatment, will result in significant thermal degradation (73%) of the total amount of glucosinolates.
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1. Introduction

Glucosinolates are a group of plant secondary metabolites found exclusively in dicotyledonous plants. The highest concentrations are found in the Brassicaceae family. The Brassicaceae family comprises many commonly consumed vegetables, condiments, forages and oil containing plants, such as cabbage, broccoli, cauliflower, Brussels sprouts and rape. Over 120 different glucosinolates have been identified to this date. According to their structure they can be classified as aliphatic, aromatic, ω-methylthioalkyl and heterocyclic (e.g., indole) glucosinolates (Fahey, Zalcmann, & Talalay, 2001). Glucosinolates and their breakdown products are of particular interest in food research because of their alleged anticarcinogenic properties. There are clear indications that they block tumour initiation by modulating the activities of Phase I and Phase II biotransformation enzymes and suppress tumours by apoptosis (Mithen, Dekker, Verkerk, Rabot, & Johnson, 2000).

Glucosinolates are responsible for the characteristic flavour and odour of Brassica vegetables (Das, Tyagi, & Kaur, 2000). The glucosinolates sinigrin and progoitrin have been found to be related to bitterness in

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Brussels sprouts (Van Doorn et al., 1998), while in cooked cauliflower glucosinolates neoglucobrassicin and sinigrin were found responsible for the bitter taste (Engel, Baty, Le Corre, Souchon, & Martin, 2002).

Myrosinase, or thioglucoside glucohydrolase EC 3.2.3.1, is the trivial name for the enzyme (or group of enzymes) responsible for the hydrolysis of glucosinolates (Fenwick & Heaney, 1983). Previous studies have localized myrosinase in the cytoplasm of specialized plant cells, myrosin cells. Autolysis or tissue damage brings myrosinase in contact with glucosinolates and hydrolysis occurs. Myrosinase activity results in the release of the glucose moiety to leave an unstable intermediate (Mithen et al., 2000), which spontaneously rearranges to produce several products. Which product is formed depends on several factors, such as pH, substrate or availability of ferrous ions (Bones & Rossiter, 1996). The products of glucosinolate hydrolysis include isothiocyanates, nitriles, thiocyanates, indoles and oxazolidinethiones; from which isothiocyanates and indoles in particular have been implicated to have anticarcinogenic properties (Mithen et al., 2000).

Many steps in the food production chain, such as cultivation, storage, processing and preparation of vegetables, may have an impact on levels and thus intake of phytochemicals (Dekker, Verkerk, & Jongen, 2000; Howard, Jeffery, Wallig, & Klein, 1997; Vos & Blijleven, 1988). Brassica vegetables are, prior to consumption, subjected to different ways of processing, domestic as well as industrial. Domestic treatments of the Brassica vegetables such as chopping, cooking, steaming and microwaving have been shown to affect the glucosinolate content considerably (Rangkadilok et al., 2002; Rodrigues & Rosa, 1999; Verkerk, Dekker, & Jongen, 2001), while the effects of industrial processes as freezing, fermenting and canning are less studied (Dekker & Verkerk, 2003; Tolonen et al., 2002).

During thermal processing of Brassica vegetables, glucosinolate levels can be reduced because of several mechanisms: enzymatic breakdown, thermal breakdown and leaching into the heating medium. In most studies on the effect of thermal processing these mechanisms are not investigated separately.

Thermal breakdown of synthetic glucobrassicin was studied by Chevolleau, Gasc, Rollin, and Tulliez (1997) and Chevolleau, Debrauwer, Boyer, and Tulliez (2002). They reported a 10% degradation of glucobrassicin after heating for 1 h at 100 °C and observed the formation of a new breakdown product, 2-(3′-indolylmethyl) glucobrassicin. Rosa and Heaney (1993) found that 10 min of boiling Portuguese cabbage was sufficient to reduce the total glucosinolate content by more than 50%. Ciska and Kozlowska (2001) cooked white cabbage for 30 min and observed the highest decrease after 5 min of cooking (35%), which gradually decreased to a 87% loss after 30 min. Simulation studies, taking into account cell lyses, enzymatic breakdown and leaching, performed by Verkerk (2002) predict considerable reduction (40–70%) of glucosinolates during cooking, mainly because of leaching into the cooking water after cell lyses has occurred.

Microwave cooking is thought to be an efficient alternative for cooking vegetables due to the low amount of cooking water required. However, according to Vallejo, Tomás-Barberán, and García-Viguera (2002), microwaving of broccoli resulted in 40% loss of Vitamin C and a 74% loss of glucosinolates. In contrast to these results, Verkerk and Dekker (2004) observed a 78% increase in total extractable glucosinolate content of red cabbage after microwave cooking for 4.8 min a 900 W, as well as the inactivation of myrosinase. These authors, however, did not use added water during microwaving, therefore, it was expected that limited leaching of nutrients took place.

Blanching and canning of foods and the effect of these thermal treatments on glucosinolates have not been well studied previously.

An earlier investigation showed a decline in available glucosinolates in canned cabbage as compared to fresh and frozen cabbage (Dekker & Verkerk, 2003). Although it is unclear what the cause for this decline was, it was most likely due to either thermal and/or enzymatic degradation. Canned vegetables undergo a substantial heat treatment; therefore the thermal degradation of glucosinolates is thought to be the most important mechanism.

The aim of this study was to investigate the thermal degradation of individual glucosinolates within the plant matrix. To study the degradation kinetics, cabbage samples underwent different temperature treatments for various times. With the results of these experiments, kinetic parameters have been estimated for the degradation. In order to study only the thermal degradation, myrosinase was inactivated to rule out the influence of enzymatic breakdown. The kinetic parameters of the individual glucosinolates determined are discussed in relation to the differences in their chemical structure.

2. Materials and methods

2.1. Materials

Three batches of refrigerated pre-chopped red cabbage (*Brassica oleracea* L. var. *Capitata* f. *rubra* DC) (size approximately 3 mm × 2 cm) were purchased from local supermarkets (C1000 and Albert Heijn, Wageningen, the Netherlands).

2.2. Sample preparation

Part of the cabbage was analyzed fresh while most of the samples were microwaved in order to inactivate

myrosinase as described by Verkerk (2002). The chopped cabbage was divided into portions of 300 g, placed in a 1000 ml glass beaker and cooked in a microwave oven at 2450 MHz (Daewoo, Model KOC-87-T, Korea). Each portion was heated for 4 min and 48 s at 900 W. After the microwave treatment the cabbage was cooled on ice. The samples were frozen immediately with liquid nitrogen and the frozen material was ground to a fine powder with a Waring blender (model 34BL99, Dynamics Corp. of America, New Hartford, CT, USA). The powder was stored at −20 °C until further analyses. Part of the fresh and microwaved cabbage was taken as a sub-sample for the preparation of a juice for the analysis of hydrolytic myrosinase activity.

2.3. Myrosinase activity

Fresh and microwave treated red cabbage was blended with a commercial liquidizer (Braun, type 4290), and sieved to remove solid particles. The filtrate (or juice) was incubated for 1 h at 40 °C in a water bath, to allow for myrosinase-catalyzed hydrolysis of endogenous glucosinolates. Part of the filtrate was incubated for 15 min at 100 °C, to inactivate the myrosinase. This part was used for dilution purposes. To 5 g of 50-fold diluted cabbage juice, 1 ml of 6 mM sinigrin was added. The juice was incubated at 40 °C for 0, 5 and 20 min. Myrosinase activity was measured by the extent of hydrolysis of a known amount of sinigrin added to the cabbage juice. The sinigrin content of the juice at different incubation times was determined by high performance liquid chromatography (HPLC) analyses.

2.4. Thermal breakdown

Frozen cabbage powder (5 g) was transferred to glass tubes (Schott GL18, diameter = 13 mm) with screw caps and was held on ice until heating. A thermocouple was placed in one tube, through the cap, to monitor the cabbage temperature. The time–temperature combinations used for heating are shown in Table 1. The tubes were heated in a heating block (Grant QBT4, Cambridge, UK). The come-up-times were between 2 and 13 min, this time was excluded from the kinetic parameter analysis. After heating the samples were cooled on ice and subsequently analyzed.

2.5. Glucosinolate analysis

2.5.1. Extraction

The method described by Verkerk et al. (2001), based on extraction of glucosinolates with hot methanol as a solvent was used with minor modifications. The heated cabbage was transferred from the heating tube by adding 12 ml hot methanol (100%), followed by shaking, and pouring the mixture into a 50 ml Greiner tube. One ml of 3 mM glucotropaeolin was immediately added as an internal standard. Samples were incubated in a water bath of 75 °C for 25 min, during which the samples were mixed several times. After incubation the samples were centrifuged 10 min at 5000*g*. The supernatant was collected in new 50 ml Greiner tubes. The pellet was re-extracted twice with 10 ml of 70% hot methanol, centrifuged and the supernatants were combined with the first supernatant.

Table 1
Experimental design for heating times (min) and temperatures

80 °C	90 °C	100 °C	110 °C	118 °C	120 °C	123 °C
5	5	1	0.5	5[a]	0.5	5[a]
30	15	5	1	10[a]	1	10[a]
60	30	15[b]	2.5	20[a]	2.5	20[a]
90	45	30[b]	5	40[a]	5	40[a]
120	60	45	7.5[b]	60[a]	10	60[a]
150	90	60[b]	10	120[a]	15	120[a]
180	120	90	15[b]		20	
210	150	120[b]	30[b]		30	
240	180	150	45		60	
300	240	180	60[b]			
		240[a]	120[b]			
		360[a]	240[a]			

[a] Performed in duplicate.
[b] Performed in triplicate.

2.5.2. Purification and desulfation

The extracted glucosinolates were purified on a 1.5 cm DEAE Sephadex A-25 anion exchange column. The column was washed twice with 1 ml millipore water, loaded with 2 ml of the glucosinolates extract and then washed twice with 1 ml of 20 mM NaAc-solution. Sulphatase enzyme (75 μl and 25 mg/ml) was added to the column and it was incubated overnight at room temperature. The second day, the desulfoglucosinolates were eluted with millipore water (3 × 0.5 ml). The eluate was filtered over a 0.45 μm filter (13 mm, Alltech, Deerfield, IL, USA) and then the sample was ready for HPLC analysis.

2.5.3. HPLC analyses

The glucosinolates in the fresh and heated cabbage and the cabbage juice were analyzed using high performance liquid chromatography as described by Verkerk et al. (2001). The HPLC method for determining desulfated glucosinolates provides a simple means for obtaining information on the glucosinolate profiles.

The desulfoglucosinolates were separated using a Nova Pak C18 (5 μm) reverse phase column (3.9 mm × 159 mm; Waters, Milford, MA, USA) with a flow rate of 1 ml/min. Elution of desulfoglucosinolates from the HPLC column was performed by gradient system of water (A) and acetonitril/water (20:80, v/v, B).

The total running time was 31 min with a gradient as follows: 100% A and 0% B for 1 min, then in 20 min to 0% A and 100% B, and in 5 min back to 100% A and 0% B.

An UV detector was used at a wavelength of 229 nm. The results were analyzed using the ChromQuest program (Thermo Electron Corporation). The desulfoglucosinolates were identified with standards of sinigrin, glucotropaeolin and by using the typical glucosinolate pattern in rapeseed samples, and expressed as μmol/100 g fresh matter. The certified reference material used was BCR-367 R, rapeseed (Linsinger, Kristiansen, Beloufa, Schimmel, & Pauwels, 2001). Glucosinolates that were identified included: glucoiberin (3-methylsulfinylpropyl), progoitrin, (2-hydroxy-3-butenyl), sinigrin (2-propenyl), glucoraphanin (4-methylsulfinylbutyl), gluconapin (3-butenyl), 4-hydroxyglucobrassicin (4-hydroxy-3-indolylmethyl), glucobrassicin (3-indolylmethyl) and 4-methoxyglucobrassicin (4-methoxy-3-indolylmethyl). The first 5 glucosinolates (e.g., glucoiberin, progoitrin, sinigrin, glucoraphanin and gluconapin) are classified as aliphatic glucosinolates, while the last three are classified as indole glucosinolates. Glucosinolate breakdown products were not measured. The total glucosinolates level was determined by adding up all individual glucosinolates, as was done by Linsinger et al. (2001) for the certification of the total glucosinolates levels in rapeseed.

2.6. Statistics and modeling

Significance was determined by performing a Student's t test in Microsoft Excel. Reaction kinetics modeling and parameter estimations were done by integral fitting of the data sets using the determinant criterion (Stewart, Caracotsios, & Sorensen, 1992). Integral fitting implies that the data sets from different incubation temperatures for each compound were fitted simulatanuously to the degradation parameters. The software package Athena Visual Workbench (www.athenavisual.com) was used for numerical integration of differential equations as well as parameter estimation of the rate constants in the differential equations following minimization of the determinant in order to obtain the reaction kinetic parameters (rate constant k_d, min^{-1}, and activation energy E_a, J/mol).

Thermal degradation of the individual glucosinolates (GS_i) was described with a first-order reaction mechanism

$$\frac{d[GS_i]}{dt} = -k_{d,i}[GS_i]. \quad (1)$$

The concentrations of glucosinolates ($[GS_i]$) were expressed as μmol/100 g FW. For the parameter estimation the individual measured concentrations were used instead of mean values of duplicate or triplicate experiments, thus taking into account all the variability in the samples. Temperature dependence of the reaction rate constants was described by the rearranged Arrhenius equation

$$k_d = k_{d,ref} \exp\left\{\left(\frac{E_a}{R}\right)\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right\}. \quad (2)$$

The temperature of 110 °C was used as the reference temperature (T_{ref} = 383 K). Estimation of $k_{d,ref}$ and E_a of each glucosinolate was done by integral fitting of Eqs. (1) and (2) simultaneously to the experimental data of all times and temperatures investigated, for each glucosinolate separately. The initial concentrations were estimated by the fitting procedure as well, to allow for uncertainty in the experimental observation at $t = 0$. T is the sample temperature (K) and R the universal gas constant (8.32 J/mol K).

3. Results and discussion

3.1. Myrosinase inactivation by microwave treatment

To discriminate the effect of thermal breakdown of glucosinolates from that of enzymatic breakdown, the endogenous myrosinase was first inactivated by microwave treatment. Verkerk and Dekker (2004) have shown that microwaving of cabbage for 4 min and 48 s effectively inactivates myrosinase. To check for inactivation of myrosinase its activity was determined in juices prepared from fresh and microwave treated red cabbage samples.

Glucosinolates were measured in three different batches of fresh and microwaved cabbage. The glucosinolate levels showed large variation as was reported in previous studies (Rosa, 1997; Ciska, Martyniak-Przybyszewska, & Koslowska, 2000). This may be attributed to biological as well as to experimental variability. Our results show that in all cases, microwaving fresh cabbage resulted in higher extractable levels for all eight individual glucosinolates, as illustrated in Fig. 1. However, due to the variability of the different batches of cabbage, these differences were not significant. This increase following microwaving was also observed by Verkerk and Dekker (2004), who found a 78% higher total extractable glucosinolate levels in red cabbage after microwaving. In the current study, a 35% increase in total extractable glucosinolates was observed after microwave treatment of red cabbage. Individual glucosinolates increased from 82% in glucobrassicin to a loss of 5% in sinigrin, following microwave treatment. Verkerk and Dekker (2004) explained this phenomenon by an increase in chemical extractability from the plant tissue after heating.

In contrast to our results, Vallejo et al. (2002) observed that microwave cooking of broccoli resulted in

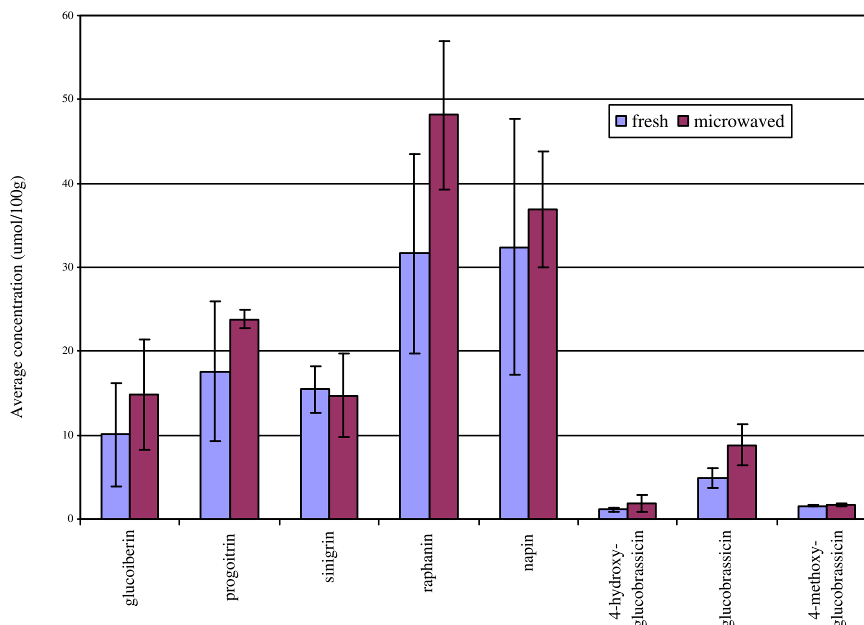

Fig. 1. Glucosinolate content in fresh and microwaved red cabbage (error bars indicate standard deviation, $n = 6$).

a decrease of 74% in total glucosinolate level. However, the use of broccoli instead of red cabbage, the use of added water and the more intense microwaving conditions (150 g broccoli and 150 g water, 5 min at 1000 W) may explain the observed differences.

3.2. Thermal degradation and modeling

Thermal degradation of glucosinolates was studied at temperatures ranging from 80 to 123 °C. Microwaved red cabbage was used as the starting material for the heating experiments. Significant breakdown of glucosinolates was observed in many of the treatments used in this study, especially in red cabbage heated at temperatures higher than 110 °C. As an example, comparison of the HPLC-profiles of glucosinolate in heated red cabbage after 5.0 (A), 40 (B) and 120 min (C) at 118 °C are shown in Fig. 2. These profiles demonstrate a profound reduction in glucosinolates due to the heat treatment.

Observed concentration profiles for the analyzed glucosinolates as a function of incubation temperature and time are shown in Fig. 3. Only limited degradation was observed at lower temperatures ($T < 110$ °C) for most glucosinolates. Indole glucosinolates showed more degradation than aliphatic glucosinolates at lower temperatures. Higher temperatures ($T > 110$ °C) resulted in significant degradation of all identified glucosinolates. All the experimental data derived from the thermal treatments (see Table 1 for conditions) was used simultaneously to determine the kinetic parameters ($k_{d,110\,°C}$ and E_a) using Eqs. (1) and (2). The kinetic parameters and their confidence intervals for the degradation of glucosinolates are summarized in Table 2.

3.3. Individual glucosinolates

All glucosinolates fit well to the assumed first-order degradation kinetics with Arrhenius type of temperature dependency, with the exception of 4-hydroxyglucobrassicin. The fitted profiles of 4-hydroxyglucobrassicin showed some consistent underestimation of the breakdown at higher temperatures (Fig. 3). This may indicate that the assumed first-order degradation mechanism does not describe the reaction mechanism for this species well. Possibly two parallel degradation reactions exist with different reference rate constants and different activation energies. To check this hypothesis a new parameter estimation was performed for degradation mechanisms with two different reaction pathways. The resulting kinetic parameters and their 95% confidence interval are $k_{d1,110\,°C} = 1.0 \times 10^{-2}$ ($\pm 0.3 \times 10^{-2}$) min^{-1}, $k_{d2,110\,°C} = 4.4 \times 10^{-2}$ ($\pm 0.7 \times 10^{-2}$) min^{-1} and E_{a1} = 0 kJ/mol, E_{a2} = 108 (±28) kJ/mol.

Bayesian statistics were used for calculating the posterior probability of both possible reaction pathway models to discriminate between both models for degradation of 4-hydroxyglucobrassicin. From these values the probability share of each model were calculated (Stewart, Shon, & Box, 1998). This resulted in a probability share for the 1-pathway model of 0.005 and for the 2-pathway model of 0.995. Based on this analysis of our experimental findings it is therefore more likely that 4-hydroxyglucobrassicin is degraded by two different reactions compared to only one reaction. The absence of a

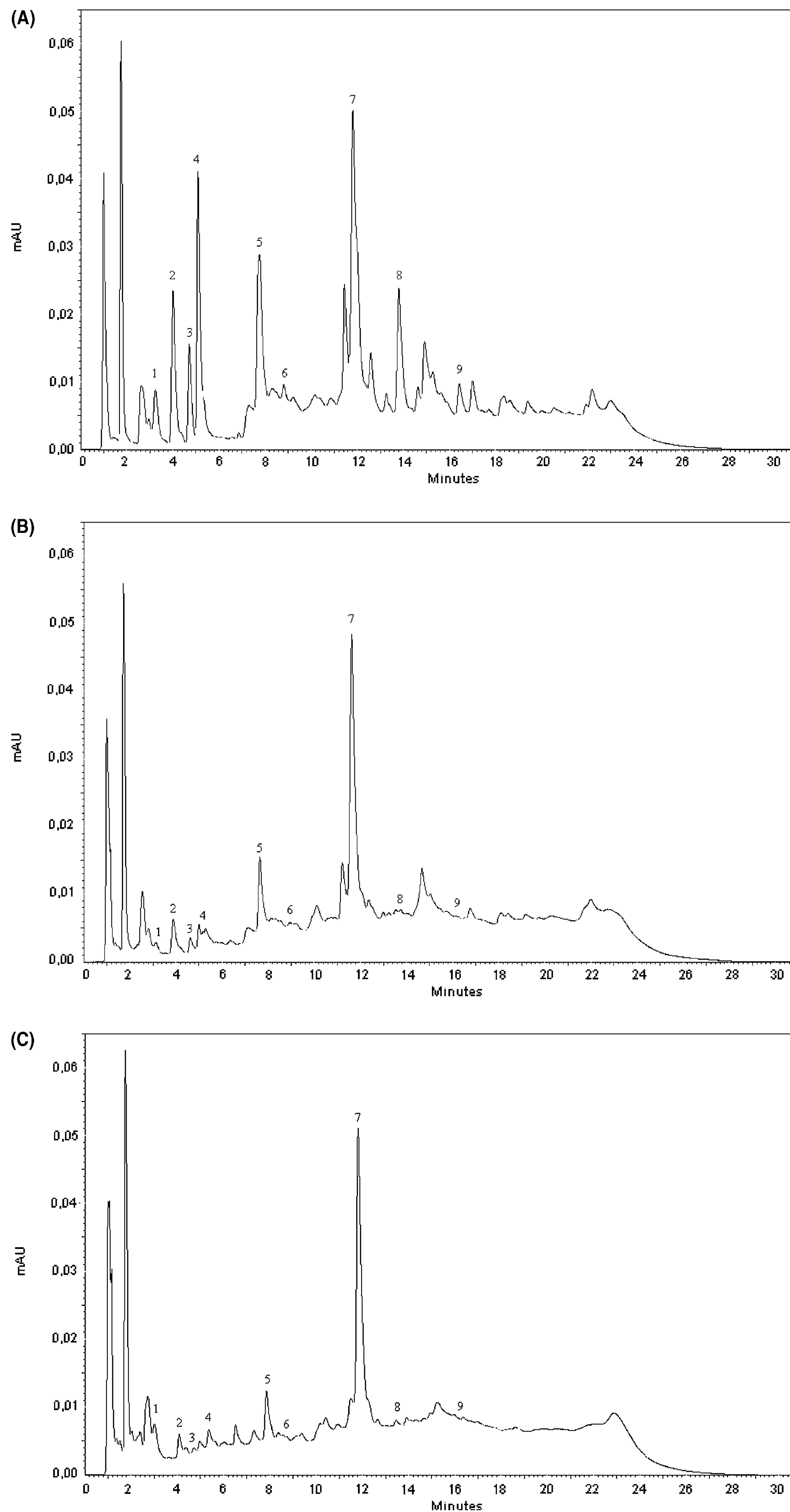

Fig. 2. HPLC chromatograms (229 nm) of the thermal breakdown of glucosinolates after (A) 5, (B) 40, (C) 120 min at 118 °C. Identified glucosinolates are: (1) glucoiberin, (2) progoitrin, (3) sinigrin, (4) glucoraphanin, (5) gluconapin, (6) 4-hydroxyglucobrassicin, (7) glucotropaeolin (internal standard), (8) glucobrassicin, (9) 4-methoxyglucobrassicin.

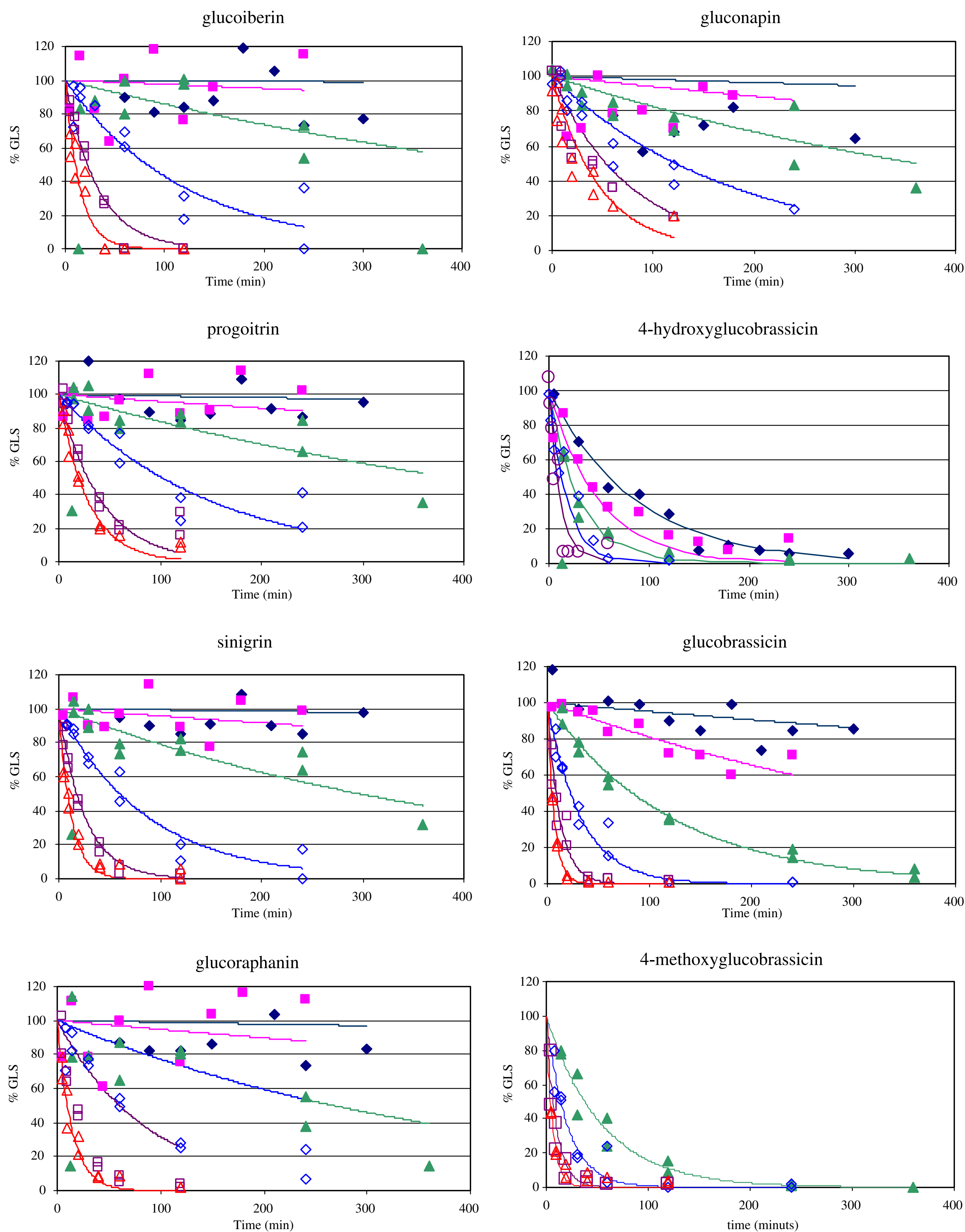

Fig. 3. Thermal degradation of glucosinolates at different temperatures: 80 (◇), 90 (■), 100 (▲), 110 (◆), 118 (□) 120 (¤) and 123 °C (△). The lines give the fitted degradation profiles by the model.

Table 2
First-order thermal degradation parameters for individual glucosinolates

	$k_{d,110\,°C}$ ($\times 10^{-3}$ min^{-1})	E_a (kJ/mol)
Glucoiberin	8.5 ± 0.5	203 ± 7
Progoitrin	6.8 ± 0.3	160 ± 5
Sinigrin	11.6 ± 0.3	190 ± 3
Glucoraphanin	11.5 ± 0.5	177 ± 5
Gluconapin	5.7 ± 0.3	129 ± 6
4-Hydroxyglucobrassicin*	54.7 ± 1.7*	58 ± 2*
Glucobrassicin	30.7 ± 0.3	155 ± 1
4-Methoxyglucobrassicin	48.7 ± 3.5	115 ± 5

Reference temperature is 110 °C. Intervals shown are 95% confidence interval.

All individual glucosinolates have been observed for 98 temperature–time combinations.

* Degradation of 4-hydroxyglucobrassicin is more accurately described by two parallel thermal degradation reaction pathways, see text for these parameters.

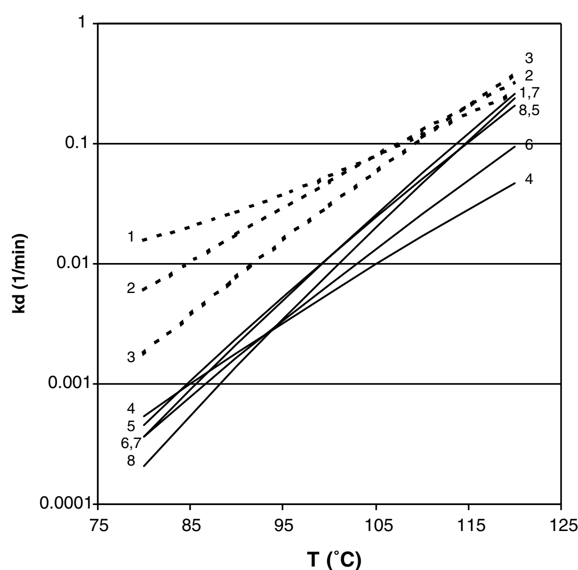

Fig. 4. Temperature effect on the degradation rate constant for the individual glucosinolates. (—–) aliphatic glucosinolates; (- - -) indole glucosinolates; individual glucosinolates: (1) 4-hydroxyglucobrassicin, (2) 4-methoxyglucobrassicin, (3) glucobrassicin, (4) gluconapin, (5) glucoraphanin, (6) progoitrin, (7) sinigrin, (8) glucoiberin.

temperature dependence of one of the pathways (E_{a1} = 0 kJ/mol) is however surprising and needs further investigation.

In Fig. 4 the temperature dependence of the degradation rate constants of the individual glucosinolates is shown graphically. The order of thermostability of individual glucosinolates, from lowest to highest k_d value at 80 °C is: glucoiberin < progoitrin ≈ sinigrin < glucoraphanin < gluconapin < glucobrassicin < 4-methoxyglucobrassicin < 4-hydroxyglucobrassicin. At 120 °C due to the differences in the activation energies the order changes to: gluconapin < progoitrin < glucoraphanin < glucoiberin < 4-hydroxyglucobrassicin ≈ sinigrin < 4-methoxyglucobrassicin < glucobrassicin. The variation at 80 °C between the most and least stable glucosinolate is much higher than at 120 °C: illustrated by a 75-fold difference in reaction rates at 80 °C compared to only an 8-fold difference at 120 °C. The thermal degradation profiles illustrated in Figs. 3 and 4, show that 4-methoxyglucobrassicin and 4-hydroxyglucobrassicin are the most thermolabile glucosinolates, especially at temperatures ⩽100 °C. In the literature only a small degree of qualitative information is available on the thermal stability of glucosinolates. Jensen, Liu, and Eggum (1995) also found 4-hydroxyglucobrassicin to be the most thermolabile of all of glucosinolates they evaluated in rapeseed meals. Rosa and Heaney (1993) found more unaccountable losses during cooking of glucoiberin, 4-methoxyglucobrassicin and neoglucobrassicin, suggesting that these glucosinolates were more thermolabile than other glucosinolates. Vallejo et al. (2002) found similar losses for both glucosinolates in broccoli, where glucobrassicin was more thermolabile than glucoiberin and glucoraphanin. These studies however, did not include 4-hydroxyglucobrassicin in their analysis. In our research, glucobrassicin was also identified as more thermolabile than glucoiberin and glucoraphanin, but 4-hydroxyglucobrassicin and 4-methoxyglucobrassicin were found to be the most thermolabile glucosinolates.

Chevolleau et al. (1997) studied the thermal breakdown of synthetic glucobrassicin (GBS). They found 10% breakdown of glucobrassicin after 1 h at 100 °C. Chevolleau et al. (2002) isolated a new thermal breakdown product of glucobrassicin, 2-(3′-indolylmethyl) glucobrassicin. None of these studies report quantitative kinetic data on the breakdown, the qualitative order of thermostability of the investigated glucosinolates is similar to our findings.

3.4. Aliphatic vs. indole glucosinolates

The inactivation rate constants of the indole glucosinolates are significantly higher ($P < 0.01$) than that of the aliphatic glucosinolates for temperatures ⩽110 °C. As can be concluded from Fig. 4 the differences in degradation rates between the two structurally different groups of glucosinolates is reduced at higher temperatures. At 120 °C both indole and aliphatic glucosinolates have very similar degradation rates, with the exception of progoitrin and gluconapin that have significantly lower degradation rates at this temperature. The indole glucosinolates comprise only a small part (8%) of the total glucosinolates level measured in red cabbage. 4-Hydroxyglucobrassicin and 4-methoxyglucobrassicin were determined to be the most thermolabile glucosinolates studied, and glucobrassicin also showed a significant degradation. These indole glucosinolates showed higher degradation rates than the aliphatic glucosinolates identified in this study. Ciska and Kozlowska (2001) also found that losses of indole GLS in cabbage (e.g., gluco-

Table 3
Predicted effects of three different heat treatments (blanching, cooking and canning) on the residual percentage of glucosinolates in the cabbage as a result of thermal degradation

	Initial concentration set to 100% (μmol/100 g FW)	Blanching (3 min, 95 °C) (%)	Cooking (40 min, 100 °C) (%)	Canning (40 min, 120 °C) (%)
Glucoiberin	14.8	100	94	18
Progoitrin	23.8	100	93	38
Sinigrin	14.7	100	91	12
Glucoraphanin	48.2	100	90	15
Gluconapin	36.9	100	93	53
4-Hydroxyglucobrassicin	1.9	93	26	3
Glucobrassicin	8.8	99	72	1
4-Methoxyglucobrassicin	1.6	97	48	1
Total aliphatic gls	138.4	100	92	29
Total indole gls	12.34	98	62	2
Total gls	150.8	100	89	27

brassicin and 4-methoxyglucobrassicin) were higher during cooking for 30 min than those of aliphatic glucosinolates (glucoiberin, progoitrin and sinigrin). They proposed that these compounds diffused to a higher extent into the cooking water. This is in accordance with findings of Vallejo et al. (2002); they observed that high pressure and conventional boiling, due to leaching into the cooking water, led to a significant loss of glucosinolates and vitamin C. Slominski and Campbell (1989) reported a substantial decomposition of indole glucosinolates after a heat treatment of cabbage at 100 °C for varying time periods, up to 50 min. They quantified the decomposition of indole glucosinolates subjected to heat treatment as the release of thiocyanate ion (50%) and production of indole acetonitriles (30%). Goodrich, Anderson, and Stoewsand (1989) blanched broccoli and Brussels sprouts and found significant losses in most glucosinolates with steam blanching, and even more with water blanching of broccoli. In Brussels sprouts, these authors found that blanching did not reduce the major glucosinolates significantly, only the indole glucosinolates 4-hydroxy- and 4-methoxyglucobrassicin showed a significant decrease.

Our study allows for a true interpretation of the thermal degradation of glucosinolates, without the confounding effect of leaching into the cooking water. It can be concluded that the quantitative results of our study on the differences between thermal degradation of indole and aliphatic glucosinolates are qualitatively in agreement with literature data.

3.5. Processing simulations

The model and the kinetic parameters for all identified glucosinolates in red cabbage was used to predict the effects of standardized conditions for blanching, cooking and canning on all individual glucosinolates (Table 3). In this prediction, only losses due to thermal breakdown are given, e.g., losses due to leaching into the blanching, cooking or canning water have not been included.

The heating conditions that were assumed for the different treatments were as follows: blanching-3 min at 95 °C, cooking-40 min at 100 °C and canning-40 min at 120 °C. These settings of constant temperature treatment are used for the simulation; they would not be seen in practice where a dynamic temperature–time profile will occur. In real treatments with a known temperature–time profile the model can be used to estimate the thermal degradation of glucosinolates. The simulation will, however, give a good indication of what will happen during blanching, cooking and canning. The results show a mild heat treatment, such as blanching, has little impact on the glucosinolates. Conventional cooking does not affect the aliphatic glucosinolates significantly; the indole glucosinolates, however decreased to a higher extent (38%). The more severe heat treatment, e.g., canning, severely affects all glucosinolates (73%), and therefore will have a great impact on the health promoting compounds available in canned Brassica vegetables. Since aliphatic glucosinolates contribute mostly to the characteristic taste of Brassica vegetables, blanching and cooking will have little impact on the taste, while canning is expected to have a large impact on the taste of the vegetables. The predicted low glucosinolate levels in canned red cabbage are in good agreement with the reported analysis on the level of glucosinolates in various consumer products (Dekker & Verkerk, 2003).

In this simulation experiment the thermal breakdown of glucosinolates was studied as an isolated process, without the confounding effect of leaching of the compounds into cooking water, therefore it is difficult to compare these values with literature data, which often combines these two phenomena in the experimental set-up.

The losses of glucosinolates observed in literature are substantially higher than the predicted values after a similar heat treatment (cooking) in present study (Table 3). Higher losses in these studies will very likely be due to leaching of components in the cooking water used and possibly enzymatic degradation of glucosinolates.

4. Conclusions

Red cabbage was heated at different temperatures for different time periods. Only thermal breakdown was investigated in this study, losses due to enzymatic degradation or leaching of glucosinolates in cooking water were eliminated. All glucosinolates showed degradation upon heating of red cabbage. The indole glucosinolates 4-hydroxyglucobrassicin, 4-methoxyglucobrassicin and to a lesser extent glucobrassicin showed a high degree of degradation, even at lower temperatures ($T \leqslant 110$ °C). At these temperatures these indole glucosinolates were more thermolabile than aliphatic glucosinolates. At higher temperatures ($T > 110$ °C) the differences in stability became much less and all glucosinolates showed substantial breakdown. The estimated degradation kinetics were used to predict the effects of standard heating conditions (blanching, cooking, canning) on the thermal breakdown of all individual glucosinolates. A mild heat treatment, such as blanching does not affect glucosinolates significantly. A more severe heat treatment, such as cooking, degrades only the indole glucosinolates significantly (38%), as compared to aliphatic glucosinolates (8%). Canning, the most severe heat treatment, degrades all measured glucosinolates (73%), thereby having a substantial effect on the health promoting potential of the glucosinolates in canned Brassica vegetables.

The thermal degradation model presented and the parameters obtained combined with models and parameters for leaching and enzymatic processes during different treatments will enable the optimisation of industrial processes with respect to an enhanced health promoting potential of processed products.

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