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Kinetics of Changes in Glucosinolate Concentrations during Long-Term Cooking of White Cabbage (*Brassica oleracea* L. ssp. *capitata* f. *alba*)

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Brassica vegetables are the predominant dietary source of glucosinolates (GLS) that can be degraded in the intestinal tract into isothiocyanates, which have been shown to possess anticarcinogenic properties. The effects of pilot-scale long-term boiling on GLS in white cabbage (*Brassica oleracea* L. ssp. *capitata* f. *alba* cv. 'Bartolo') was experimentally determined and mathematically modeled. Cabbage was boiled, resulting in a dramatic decrease of 56% in the total GLS levels within the plant matrix during the first 2 min. After 8–12 min of boiling, the decrease progressed to over 70%. Progoitrin had an exceptionally higher decline rate in comparison to all other GLS. As boiling progressed the concentration of all GLS continued to decrease at a lower rate for the remaining cooking period. A mathematical model was used to describe the concentration profile of the GLS in the plant matrix, based on leaching of GLS to the water phase due to cell lysis and thermal degradation of the GLS both in the plant matrix and in the water phase. The model described the concentration profiles very well. Estimated lysis and degradation rate constants for white cabbage differed from those reported in the literature for red cabbage. The degradation rate constants found were significantly higher in the plant matrix when compared to those in the water phase for all GLS. Identification of the kinetics of decline of GLS during cooking can aid in designing processing and preparation methods and determining the conditions for the optimal effects of ingestion of Brassicaceae toward cancer prevention.

KEYWORDS: Glucosinolates; cabbage; *Brassica oleracea* L. ssp. *capitata*; processing; rate constants; mathematical modeling

INTRODUCTION

Glucosinolates (GLS) are β-thioglucoside *N*-hydrosulfates intrinsic to Brassicaceae, for example, red and white cabbage, cauliflower, kale, broccoli, and Brussels sprouts. A dozen different GLS are normally found in each species, and approximately 120 different GLS have been identified in nature (*1*). The GLS are subcategorized according to their chemical structure in aromatic, aliphatic, and indole GLS. GLS coexist with, but are physically separated from, a plant thioglucosidase (myrosinase) within a myrosinase−glucosinolate system (*2*). Damaging of plant tissue by handling, processing, or chewing will rupture cellular membranes and allow contact between GLS and myrosinase, releasing a complex variety of breakdown products including isothiocyanates (*3*).

The interest in GLS from a human health perspective is due to the indications that GLS breakdown products may have anticarcinogenic properties (*4*). To make reliable estimates of the levels of GLS ingested by humans, and thereby apply epidemiological studies to search for effects of inverse cancer risk on humans, it is imperative to be able to assess and subsequently predict how processing conditions (domestic or industrial) affect the inherent levels in the products that are actually consumed.

Vegetables are frequently subjected to various forms of treatments to make them more suitable for human consumption as well as to enhance their storage capabilities. Many types of treatments influence the cellular integrity, which ultimately affects the contents of phytochemicals. Conventional processing methods such as boiling and blanching of *Brassica* vegetables have been shown to affect the content of GLS by different processes: (i) leaching of GLS in cooking water, (ii) enzymatic hydrolysis by myrosinase, and (iii) thermal degradation of GLS (*5*). Rosa and Heaney showed a decrease of 50% in the individual and total GLS concentrations in four types of

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Portuguese cabbage and one hybrid white cabbage when cooked for 10 min (*6*). Ciska and Kozłowska cooked cabbage for 5–30 min and found a gradual decrease of the GLS content of the cabbage with the major losses occurring during the first few minutes (*7*). Mullin and Sahasrabudhe found that 10–30% of the GLS in various cabbage types were leached into the water after 15–20 min of cooking (*8*). Wennberg investigated the effects of blanching (5 min in boiling water) on white cabbage and found losses of 74 and 50% for the total GLS in two different cultivars investigated (*9*).

Bones and Rossiter recently reviewed the thermal degradation studies on GLS in model systems, indicating relative high degrees of degradations as functions of temperature (>100 °C) and time (several hours) (*10*). They also pointed out that there had been performed surprisingly few fundamental studies regarding the effects of processing on GLS.

As GLS are the subject of much interest and research, it is of significance to investigate the effects of processing on cabbage to obtain a deeper understanding of the resultant GLS levels. The present study shows the effect of boiling white cabbage on the levels of individual and total GLS using pilot-scale equipment. The long cooking times do not represent common domestic practices, but were chosen to study the degradation kinetics during thermal treatment of cabbage at various processing times.

MATERIALS AND METHODS

Chemicals. Methanol and acetonitrile were obtained from Merck KGaA (Darmstadt, Germany). Glucotropaeolin (benzylglucosinolate), sinigrin (prop-2-enylglucosinolate), glucoraphanin (4-methylsulfinylbutylglucosinolate), and glucoiberin (3-methylsulfinylpropylglucosinolate) were purchased from C_2 Bioengineering (Karlslunde, Denmark). Trifluoroacetic acid (TFA, 1 mL ampules) was obtained from Fluka Chemie GmbH (Buchs, Switzerland). Methanol with 0.1% TFA was supplied by Riedel-de Haën/Honeywell Specialty Chemicals Seelze GmbH (Hanover, Germany). Liquid nitrogen and helium gas were supplied by Hydro Gas and Chemicals AS (Oslo, Norway). All chemicals and gases used were of analytical grade.

Plant Material and Wet-Thermal Experiment. White cabbage (*Brassica oleracea* L. ssp. *capitata* f. *alba* cv. 'Bartolo') was cultivated at the Vollebekk testfield in Aas, Akershus, Norway, and stored for <1 day at 0–1 °C at high relative humidity prior to large-scale processing. Cv. 'Bartolo' is frequently used by the vegetable-processing industry and is a late-season hybrid with a round, dense head, a short core, and many outer leaves; it was developed for long-term storage and is relatively tolerant of pepper spot and gray spot. A random representative selection of the cabbages was chosen and stripped of their outer leaves and divided in four similar pieces; the central core was removed, and the pieces were shredded to a 3 mm width size using an industrial shredder (Eillert B.V. Ulft, The Netherlands). Raw shredded white cabbage was sampled and used as reference to unprocessed material. Thermal treatment was carried out three times consecutively. Each batch implemented 45 kg white cabbage (approximately equivalent to 18 heads) and approximately 50 L of boiling tap water in a thermostated, jacketed pilot-plant scale NOVLAB 100 L boiler (NOVLAB AB, Malmö, Sweden) with mild alternating stirring. The ratio of vegetable to water was maintained throughout the course of the experiment by adding water to compensate for evaporation. Samples of approximately 30 g of cabbage were taken at 0, 2, 4, 8, 12, and 15 min and then every 15 min until 150 min. Processing was terminated by submerging the cabbage samples in liquid nitrogen in a porcelain mortar and ground with a pestle to a coarse particle size. It was further stored in plastic containers with tight-fitting lids at −40 °C until freeze-drying in vacuo.

Glucosinolate Analysis. The analysis of intact GLS was carried out using ion-pair chromatography, negating the need for desulfation (*11, 12*). Samples were lyophilized using a Christ Gamma 1-16LSC (Martin Christ GmbH, Osterode am Harz, Germany), milled to a fine powder using a 0.75 mm sieve size Retsch ZM1 mill (Retsch GmbH & Co. KG, Haan, Germany). An amount of 4.5 mL of near-boiling 70% (v/v) methanol was added to 200 mg of sample and maintained at temperature for 3 min. The sample was then homogenized, to complete disintegration of cells and dispersion of the biomolecules, using an Ultra-Turrax T25 Basic [IKA-Werke GmbH & Co. (Staufen, Germany)] fitted with a S25N-8G dispersion tool at 21500 rpm for 1 min prior to centrifugation at 4300*g* for 15 min; the supernatant was decanted and the pellet resuspended with 3 mL of 70% methanol on a whirlmixer and then centrifuged; the supernatants were combined. Aliquots of 1.5 mL were evaporated to dryness (4 h, 45 °C) using a Savant SPD131DDA Speed Vac Concentrator (Thermo Electron Corp., Vantaa, Finland), then resolved with an appropriate amount of water, and subsequently filtered using a 0.45 μm PVDF Millex-HV (Millipore Corp., Bedford, MA). The separation and detection were performed on an Agilent 1100 series LC/MSD Trap XCT system with a photodiode array detector (Agilent Technologies, Waldbronn, Germany) using a Betasil C18 (250 × 2.1 mm, 5 μm) with a matching guard column (40 × 2.1 mm, 5 μm) from Thermo (Thermo Fisher Scientific, Inc., Waltham, MA). Injection volume was 5 μL, column flow was 0.2 mL min^{-1}, and column temperature was 25 °C. Mobile phases were (A) 0.1% TFA in water and (B) 0.1% TFA in methanol with a gradient program of 0–5 min, 100% A; 5–30 min, 0–80% B; 30–35 min, 80–100% B; 35–40 min, 100% B; and 40–42 min, 100–0% B. The column was re-equilibrated at 100% A for 3 min prior to the next run. Detection was carried out at 227 nm, and quantification was based on selected external standards according to the method of Tian et al. (*13*). The mass spectrometer were used in a negative electron polarity mode with a capillary voltage of 3500 V, a drying temperature of 350 °C, and a nebulizer pressure of 40 psi. The nitrogen drying gas flow was set at 10 L min^{-1}. The ion trap was used to facilitate the identification of the GLS on the basis of their parent and fragment ions (*14*). The ion trap software version used was LC/MSD Trap Software version 5.2 SR1. Gluconapin (3-butenylglucosinolate) was also detected, but as the inherent levels were so low, it was excluded from further study. All results are based on triplicate thermal treatments and duplicate extractions and reported as arithmetic means in micromoles per 100 g of fresh weight (FW) ± standard deviation (SD).

Mathematical Modeling. During cooking of cabbage several mechanisms can affect the level of GLS in the plant matrix: (1) enzymatic hydrolysis by the endogenous myrosinase released upon decompartimentalisation and lysis of the plant cells; (2) leaching to the water phase due to lysis of the plant cells upon heating; and (3) thermal degradation at the elevated temperatures of the cooking process. The GLS degradation rates in intact plant cells and in the cooking water may be different due to different environmental conditions (pH, ionic concentrations, etc.).

Because of the relatively fast heating of the cabbage in the already boiling water, it is assumed that myrosinase will be inactivated rapidly (*15*). Therefore, the mathematical modeling used for this process includes only the effect of lysis/leaching and thermal degradation in the plant matrix and in the cooking water.

Cell lysis is described by a first-order kinetics (eq 1) as this was also observed for the lysis of red cabbage cells by Verkerk (*16*). A mass balance is used to relate the fraction of lysed cells to the fraction of intact cells (eq 2):

$$\frac{dC_{C,i}}{dt} = -k_l C_{C,i} \quad (1)$$

$$C_{C,l} = 1 - C_{C,i} \quad (2)$$

In these equations $C_{C,i}$ is the fraction of intact cells, $C_{C,l}$ is the fraction of lysed cells, k_l is the lysis rate constant (min^{-1}), and t is time (min).

The result of cell lysis is that the lysed part of the mass of the vegetable is in direct contact with the cooking water; because it is assumed that diffusion processes are not rate limiting, the volume of

Table 1. Changes in the Concentrations (Micromoles per 100 g of FW ± SD, $n = 3$) of Aliphatic and Indole GLS during Long-Term Cooking

	aliphatic				indole				Σ^i
time (min)	IB[a]	PROG[b]	SIN[c]	RAPH[d]	4-OH-GB[e]	GB[f]	4-met-GB[g]	neo-GB[h]	GLS
0 (fresh)	90.6 ± 7.2	61.0 ± 8.8	55.8 ± 4.0	27.1 ± 2.6	4.0 ± 0.1	76.9 ± 8.2	21.5 ± 1.1	3.4 ± 0.8	340 ± 23
2	48.2 ± 0.7	4.5 ± 0.3	29.5 ± 0.9	14.6 ± 0.8	2.0 ± 0.5	39.7 ± 2.5	10.0 ± 0.4	1.5 ± 0.1	150 ± 3
4	37.7 ± 5.2	3.5 ± 1.0	22.3 ± 1.4	10.3 ± 0.9	1.5 ± 0.5	29.2 ± 2.9	7.5 ± 1.0	1.5 ± 0.4	113 ± 9
8	28.5 ± 3.6	2.0 ± 0.7	17.7 ± 0.4	8.7 ± 0.7	1.3 ± 0.4	23.1 ± 1.2	5.7 ± 0.8	1.0 ± 0.1	87.9 ± 6.9
12	26.6 ± 3.2	1.8 ± 0.6	16.7 ± 0.3	8.2 ± 0.7	1.3 ± 0.5	21.2 ± 1.0	5.3 ± 0.7	1.0 ± 0.1	82.1 ± 5.7
15	22.8 ± 2.0	1.5 ± 0.4	14.2 ± 2.5	7.2 ± 0.6	1.1 ± 0.6	17.0 ± 3.3	4.4 ± 0.6	0.8 ± 0.2	69.1 ± 9.5
30	21.4 ± 1.7	1.2 ± 0.2	13.1 ± 1.0	6.9 ± 0.5	0.9 ± 0.4	13.5 ± 1.5	4.3 ± 1.0	0.8 ± 0.1	62.0 ± 5.1
45	20.6 ± 1.9	1.0 ± 0.1	12.4 ± 0.8	6.5 ± 0.7	0.7 ± 0.4	12.0 ± 1.3	3.8 ± 1.1	0.8 ± 0.1	57.8 ± 5.1
60	19.1 ± 1.0	0.9 ± 0.1	11.1 ± 0.3	5.9 ± 0.7	0.5 ± 0.2	9.4 ± 0.9	2.9 ± 0.9	0.7 ± 0.1	50.5 ± 3.1
75	17.6 ± 1.5	0.8 ± 0.1	10.0 ± 0.8	5.3 ± 0.9	0.5 ± 0.2	7.9 ± 1.2	2.4 ± 0.9	0.7 ± 0.1	45.2 ± 5.1
90	16.3 ± 0.8	0.7 ± 0.1	9.0 ± 0.4	4.8 ± 0.8	0.4 ± 0.1	6.5 ± 1.0	1.9 ± 0.7	0.6 ± 0.1	40.3 ± 3.6
105	13.0 ± 1.2	0.8 ± 0.2	7.9 ± 0.9	4.1 ± 0.9	0.2 ± 0.0	5.4 ± 1.2	1.5 ± 0.6	0.6 ± 0.1	33.4 ± 4.8
120	12.1 ± 1.1	0.8 ± 0.2	7.2 ± 0.8	3.7 ± 0.8	0.3 ± 0.2	4.4 ± 0.9	1.2 ± 0.5	0.6 ± 0.1	30.3 ± 4.1
135	11.5 ± 1.2	0.8 ± 0.3	6.7 ± 0.9	3.5 ± 0.9	0.3 ± 0.1	3.8 ± 1.0	1.1 ± 0.5	0.6 ± 0.1	28.1 ± 4.5
150	11.0 ± 1.3	0.7 ± 0.2	6.3 ± 0.9	3.3 ± 0.8	0.2 ± 0.1	3.4 ± 0.7	0.9 ± 0.4	0.6 ± 0.1	26.4 ± 4.1

[a] Glucoiberin. [b] Progoitrin. [c] Sinigrin. [d] Glucoraphanin. [e] 4-Hydroxyglucobrassicin. [f] Glucobrassicin. [g] 4-Methoxyglucobrassicin. [h] Neoglucobrassicin. [i] Total GLS.

the "free" water phase is in fact increasing as more cells are lysed. This can be described by the following relationship (eq 3):

$$\frac{dM_w}{dt} = -\frac{dC_{C,i}}{dt}M_{v,0} = k_l C_{C,i} M_{v,0} \quad (3)$$

In eq 3 M_w is the mass of free water (g) and $M_{v,0}$ is the initial mass of vegetable (g).

According to this model the leaching of GLS is the direct consequence of the cell lysis. The GLS content of the cells that lyse is added to the free water phase. No differences in leaching behavior of individual GLS are expected according to this mechanism. To describe this mathematically, one has to take into account not only the amount of GLS from the lysing cells but also the diluting effect caused by the increase of the mass of free water caused by this lysing (eq 4):

$$\left.\frac{dC_w}{dt}\right|_L = \left(\frac{k_l C_{C,i} M_{v,0} C_v}{M_w}\right) - \left(C_w \frac{k_l C_{C,i} M_{v,0}}{M_w}\right) = \frac{k_l C_{C,i} M_{v,0}(C_v - C_w)}{M_w} \quad (4)$$

In eq 4 L refers to the fact that this is the change due to leaching only, C_w is the concentration of GLS in the free water (μmol/g), and C_v is the concentration of GLS in the intact part of the vegetable (μmol/g).

Thermal breakdown is described by first-order kinetics, similar to previous studies (*16, 17*). Because breakdown rates were observed to be different in vegetable and in cooking water, different rate constants were used (equation 5 and 6):

$$\frac{dC_v}{dt} = -k_{d,v} C_v \quad (5)$$

$$\left.\frac{dC_w}{dt}\right|_B = -k_{d,w} C_w \quad (6)$$

In these equations B refers to the fact that this is the change due to breakdown only, $k_{d,v}$ is the breakdown rate constant in vegetable (min^{-1}), and $k_{d,w}$ is the breakdown rate constant in water (min^{-1}).

All estimations of the reaction rates were performed on the respective experimentally determined individual concentrations of each long-term boiling experiment, and not by using the average values of the three consecutive runs, effectively accounting for sample variability. Mathematical modeling was performed using Athena Visual Studios v10.0 software (Stewart and Assoc. Engineering Software Inc., Madison, WI).

RESULTS AND DISCUSSION

Boiling of white cabbage at semi-industrial scale was investigated to assess the effects on total and individual GLS. In addition, the kinetic parameters for leaching and thermal degradation of individual GLS have been estimated from the observed experimental data.

HPLC analysis revealed a total of nine different GLS, namely five aliphatic and four indole (**Table 1**). Gluconapin was also detected, but as the initial levels were so low, it was excluded from further studies. Aliphatic GLS were found in the highest absolute values comprising 69% and indole GLS the remaining 31%. The observed glucosinolate profile is in agreement with previous results (*6, 18, 19*). Our results show that glucoiberin was the most abundant glucosinolate in the white cabbage used, constituting 27 and 39% of total GLS and total aliphatic contents, respectively. Glucobrassicin (3-indolylmethylglucosinolate) was the most abundant indole GLS, constituting 23 and 73% of total GLS and total indole GLS, respectively. The total GLS levels (340 μmol/100 g) determined in untreated cabbage correspond well with the findings by others; Rosa and Heaney (*6*) found 335 μmol/100 g of FW of total GLS, and Wold (*19*) determined the average levels of total GLS in the identical cultivar ('Bartolo') during two years on average to be 388 μmol/100 g of FW. Somewhat lower levels were determined by Ciska and Kozłowska (*7*) as well as Slominski and Campbell (*20*), who found 73.3 and 112 μmol/100 g of FW, respectively. Variations in cultivars, growing conditions, and environmental factors are likely to cause the observed deviations as reported by Ciska et al. (*21*). A study by Verkerk et al. on the storage of chopped white cabbage has been shown to significantly increase the levels of 4-methoxyglucobrassicin (4-methoxy-3-indolylmethylglucosinolate) and neoglucobrassicin (1-methoxy-3-indolylmethylglucosinolate) (*22*). However, as the cabbage in this study was boiled immediately after shredding, it is assumed that no such increases occurred.

The time-course effects on individual GLS in boiled cabbage are shown in **Table 1** and are illustrated for total aliphatic and indole GLS in **Figure 1**. **Figure 2** depicts how the concentrations change for sinigrin and 4-methoxyglucobrassicin, as representatives of aliphatic and indole GLS respectively, as boiling progresses. Boiling dramatically reduced the initial levels of GLS during the first 2 min by 56, 59, and 50% for the total values of GLS, alphatic, and indole, respectively. The reductions for individual aliphatic GLS ranged from 46 to 47%, with the exception of progoitrin (2-hydroxy-3-butenylglucosinolate), which was reduced 93%. Reductions in the indole GLS ranged from 48 to 56%, with the highest loss in neoglucobrassicin. The most abundant aliphatic and indole GLS, that is, glucoiberin and glucobrassicin, were reduced by 47 and 48%, respectively. At 8 and 12 min, the overall reduction was around 70% of the initial levels except for progoitrin, which then was reduced by

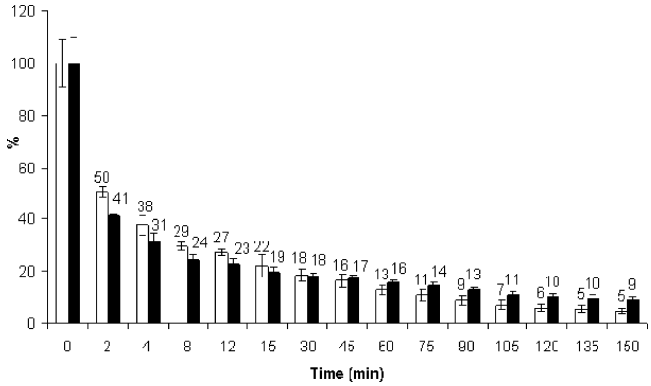

Figure 1. Relative concentrations of total aliphatic (white bars) and indole (black bars) GLS in long-term-boiled cabbage as compared to fresh. Error bars represent standard deviation. $n = 3$.

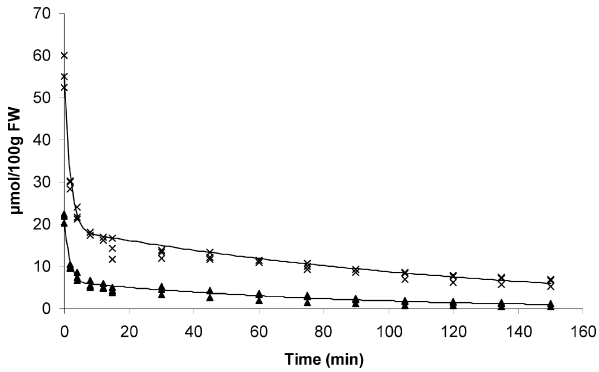

Figure 2. Example of the leaching of sinigrin (×) and 4-methoxyglucobrassicin (▲) during the course of long-term boiling of cabbage. $n = 3$. Lines fitted represent the degradation profiles as given by the model.

97%. Further reductions continued throughout the cooking period at a lower rate than initially.

In untreated cabbage intact cells GLS are effectively separated from the hydrolytic enzyme myrosinase. At cell integrity disruption myrosinase is capable of specifically converting GLS into thiocyanates, isothiocyanates, and nitriles (*2*). If, however, the cell structure remains intact until irreversible denaturation of this enzyme occurs, that is, >60 °C (*23*), then the GLS losses in the plant tissue are expected to be caused mainly by leaching and thermal degradation. Thermal degradation of enzymatic cofactors to myrosinase, such as epihiospecifier protein (ESP), occurs at even lower temperatures of around 50 °C (*24*). Degradation of GLS on the cut surface of the cabbage occurs instantaneously as the enzyme and substrate come into contact due to shredding (*5*). Heat transfer into the plant tissue is assumed to occur quickly due to the small physical size of the shredded cabbage when submerged in already boiling water. The water temperature just after immersion of the cabbage was measured to be above 60 °C, and after 60 s, it was determined to be 98 ± 2 °C.

The literature indicates divergent results for the GLS content found in processed cabbage. Wennberg et al. investigated the effects of blanching (solid/water ratio = 1:1, 5 min of boiling, 15 min of air cooling) on shredded white cabbage, finding losses for the total GLS of 50 and 74% for the two investigated cultivars (*9*). This is in reasonable accordance with our results, which indicate a decline of 67% after 4 min. However, the large reduction in progoitrin was not found in the study by Wennberg. Sones et al. (*25*) found a 28% reduction in the total GLS content in cabbage boiled for 10 min in water (the ratio of cabbage to

Table 2. Rate Constants for Aliphatic and Indole GLS (10^{-3} min^{-1} ± SD, $n = 3$)[a]

	$k_{d,v}$	$k_{d,w}$	$k_{d,lit.}$[b]
glucoiberin	155 ± 23	7.2 ± 1.0	8.5 ± 0.5
progoitrin	1457 ± 465	65.7 ± 53.6	6.8 ± 0.3
sinigrin	158 ± 20	7.7 ± 0.9	11.6 ± 0.3
glucoraphanin	157 ± 25	6.8 ± 1.0	11.5 ± 0.5
4-hydroxyglucobrassicin	144 ± 59	13.6 ± 4.3	54.7 ± 1.7
glucobrassicin	168 ± 27	15.4 ± 2.2	30.7 ± 0.3
4-methoxyglucobrassicin	212 ± 31	12.7 ± 2.0	48.7 ± 3.5
neoglucobrassicin	181 ± 56	4.9 ± 2.0	62.6 ± 1.7

[a] Rate constants are shown with 95% confidence interval. [b] $k_{d,lit.}$ values are all calculated from the rearranged Arrhenius equation using $k_{d,110\ °C}$ values as found by Oerlemans et al. (*17*), except *neo*-GB (unpublished result).

water was not reported) with 0.05% salt This is a much lower loss than that encountered in our study, which gave 74 and 76% reductions for the 8 and 12 min samples, respectively. These large deviations in results could be ascribed to the degree of exposed surface of the shredded cabbage compared to intact leaves used by Sones et al. It is not clear how much water they used. It may also be speculated that the use of salt in the cooking water might have affected overall the osmotic pressure, consequently reducing the extent of leaching. Rosa and Heaney (*6*) cooked coarsely (30–40 cm^2) chopped cabbage for 10 min (cabbage/water ratio = 1:5), reporting losses of total GLS of 53% for cabbage, which is closer to our findings, supporting the notion that the extent of cellular disruption is a major factor regarding the degree of leaching. Rosa and Heaney reported that >91% of total GLS was recovered in cooked solid material and water, which indicates a relatively low degree of unaccountable losses due to thermal and/or enzymatic degradation.

Ciska and Kozłowska (*7*) cooked white cabbage with a solid/water ratio of 1:3 for 5–30 min, also identifying the highest rate of reduction during the first 5 min. They found that the indole GLS levels had higher reduction rates compared to aliphatic likely caused by a higher affinity to leaching. This is contrary to our results. However, our results show that after 30 min, both the aliphatic and indole GLS were reduced by 82%, which is similar to the findings by Ciska and Kozłowska (*7*), who found losses of 86 and 88% of aliphatic and indole after 30 min of boiling. They also identified progoitrin having a 40% loss during the first 5 min of the cooking period. However, our study showed an even higher initial loss of progoitrin of 94% after 4 min. Ciska and Kozłowska (*7*) also indicated that glucoiberin seemed to be the most thermolabile glucosinolate due to a low degree of recovery in the water. In our experiment the water was not assessed, but the degradation rates could still be estimated by using the mathematical modeling of the GLS concentrations in the cabbage.

The results of the scaled up boiling experiment were modeled, yielding corresponding degradation rate constants in the vegetable matrix and in water as shown in **Table 2**. The effect of long-term boiling on cabbage is exemplified in **Figure 2** for one aliphatic (sinigrin) and one indole GLS (4-methoxyglucobrassicin), with the fitted lines representing the degradation profiles as given by the model. The initial drop in GLS levels could not be described by the leaching process alone. Complete leaching under the experimental conditions (weight ratio of water to cabbage of 1:1) would result in a reduction of the GLS in cabbage of around 50%. This is based on the expected distribution of GLS between the nearly equal volumes of water outside and inside the cabbage structure (composed of lysed cells). Because the observed initial drop is considerably >50%,

also (thermal) degradation of the GLS should have occurred. This degradation is initially at a much higher rate when compared to the degradation in the long run. This can be explained by assuming different degradation rate constants in the intact vegetable tissue and in the cooking water as given in **Table 2**. After complete cell lysis has occurred, the cooking water composition will be equal to the composition of the water present in the lysed cells of the cabbage. The aliphatic GLS returned rate constants in the intact vegetable cells of (155–158) $\times 10^{-3}$ min^{-1}, whereas progoitrin gave a value almost 10 times higher. Modeling was also applied by Oerlemans et al. (*17*) using red cabbage with inactivated myrosinase prior to thermal degradation experiments. They studied the breakdown at temperatures between 80 and 123 °C. Especially at the lower temperatures studied it was found that degradation was more pronounced for the indole GLS [especially 4-hydroxyglucobrassicin (4-hydroxy-3-indolylmethylglucobrassicin) and 4-methoxyglucobrassicin] compared to the aliphatic GLS. Overall, their findings indicate that GLS are relatively stable at practical temperatures and time intervals used by conventional processing methods, for example, blanching and boiling. Our results indicate higher degradation rates for indole GLS in the intact plant tissue ranging from 144 $\times 10^{-3}$ min^{-1} to 212 $\times 10^{-3}$ min^{-1}, thus somewhat higher than for most aliphatic GLS. The constants determined indicate the same trends as Oerlemans et al. (*17*) with higher lability for the indole GLS. The breakdown rates of the GLS in the cooking water appeared to be much lower. As shown in **Table 2**, they were estimated around 10−40 times lower when compared to the degradation in the intact plant tissue. The reason for this difference in stability is unclear. It could be that different pH values, or a higher concentration of certain compounds in the intact plant cells, catalyze the degradation of GLS. Another possibility is remaining activity of myrosinase, although this is not expected given the temperature profile of the process, which should lead to a more rapid denaturation of the enzyme when compared to cell lysis.

In conclusion, the results indicate that the individual and total levels of GLS present in shredded white cabbage are significantly reduced during semi-industrial scale boiling, with the majority of losses occurring during the first few minutes. The indole GLS seem to be more affected than the aliphatic GLS. Further investigations into the actual fate of the GLS regarding leaching versus actual degradation, thermal or other, should be conducted to obtain a more comprehensive understanding of the actual underlying circumstances.

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