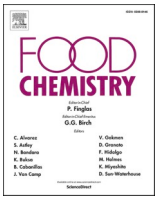
Food Chemistry 404 (2023) 134594

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem



Plant matrix concentration and redox status influence thermal glucosinolate stability and formation of nitriles in selected *Brassica* vegetable broths

Matthias Renz [a,b], Matthijs Dekker [c], Sascha Rohn [b], Franziska S. Hanschen [a,*]

[a] Leibniz Institute of Vegetable and Ornamental Crops (IGZ) e.V., Department of Plant Quality and Food Security, Theodor-Echtermeyer-Weg 1, 14979 Grossbeeren, Germany
[b] Technische Universität Berlin, Institute of Food Technology and Food Chemistry, Gustav-Meyer-Allee 25, 13355 Berlin, Germany
[c] Wageningen University & Research, Food Quality and Design Group, PO Box 17, 6700 AA Wageningen, the Netherlands

ARTICLE INFO

Keywords:
Cabbage
Brassica oleracea
Glucosinolate
Cooking
Hydrogen sulfide
Nitrile

ABSTRACT

Brassica vegetables are frequently consumed foods of nutritional interest, because they are rich in glucosinolates (GLSs). Among GLS breakdown products, especially isothiocyanates are known for their health-beneficial effects, while nitriles are less beneficial. To increase the understanding of the plant matrix's influence on GLS degradation, differently concentrated vegetable broths were prepared from selected *Brassica* vegetables (kohlrabi and red cabbage) and subsequently boiled. Altogether, heat stability and conversion of GLSs to the corresponding nitriles were both strongly influenced by vegetable type and plant matrix concentration in the broths. After boiling kohlrabi broths for 120 min, recovery of 4-(methylthio)butyl-GLS as nitrile was 55.5 % in 1 g/mL broth and 8.4 % in 0.25 g/mL broth. In follow-up experiments, a pronounced influence of the matrix's redox status was identified, with H_2S being an important factor. A better understanding of these processes will help to preserve health-promoting effects of GLSs in *Brassica* vegetables in the future.

1. Introduction

The plant order Brassicales, and especially the genus *Brassica* contain many vegetables with great importance for human nutrition, such as cabbages, broccoli, and kohlrabi. Apart from their content in vitamins and polyphenols, *Brassica* vegetables have gained a lot of scientific interest due to their content of glucosinolates (GLSs) (Dejanovic et al., 2021; Giorgetti et al., 2018). These are amino acid-derived plant secondary metabolites, consisting of an *S*-*β*-D-glucose connected to an *O*-sulfated (*Z*)-thiohydroximate function (Blazevic et al., 2020). Depending on their side chain, GLSs are commonly divided into aliphatic, benzenic, and indolic subgroups. During disruption of plant tissue, for example during cutting of vegetables, GLSs get in touch with the endogenous enzyme myrosinase (thioglucosidase, E.C. 3.2.1.147) that cleaves the glucose moiety. The instable aglycone spontaneously rearranges to form isothiocyanates (ITCs) and nitriles (Wittstock & Burow, 2010). The formation of the final product is influenced by pH, ferrous ions, and presence of additional enzymes such as nitrile specifier and epithiospecifier proteins (EPS) (Burow & Wittstock, 2009). Aliphatic GLSs with a terminal double bond may also form epithionitriles (ETNs) in presence of ESPs (Witzel, Abu Risha, Albers, Börnke, & Hanschen, 2019). The GLS core structure as well as the schematic formation of GLS degradation products are displayed in Fig. 1.

The scientific interest in GLSs is mainly due to their health-beneficial properties. Epidemiological studies have linked consumption of *Brassica* vegetables to decreased risk of developing cancer (Veeranki, Bhattacharya, Tang, Marshall, & Zhang, 2015). Further studies also identified anti-inflammatory, anti-diabetogenic, and neuroprotective properties (Sikorska-Zimny & Beneduce, 2020). These effects are strongly linked to the presence of ITCs (Mitsiogianni et al., 2019). On the other hand, the corresponding nitriles that can be formed after GLS degradation showed either no or even adverse effects (Basten, Bao, & Williamson, 2002; Kupke et al., 2016). Therefore, an optimum in conversion of GLSs towards ITCs is crucial to maintain the health-beneficial effects of *Brassica* vegetables.

It is important to consider that most *Brassica* vegetables are usually consumed after thermal processing such as boiling, steaming, or frying. This stops enzymatic degradation due to denaturation of enzymes

* Corresponding author.
E-mail address: hanschen@igzev.de (F.S. Hanschen).

https://doi.org/10.1016/j.foodchem.2022.134594
Received 15 July 2022; Received in revised form 16 September 2022; Accepted 10 October 2022
Available online 13 October 2022

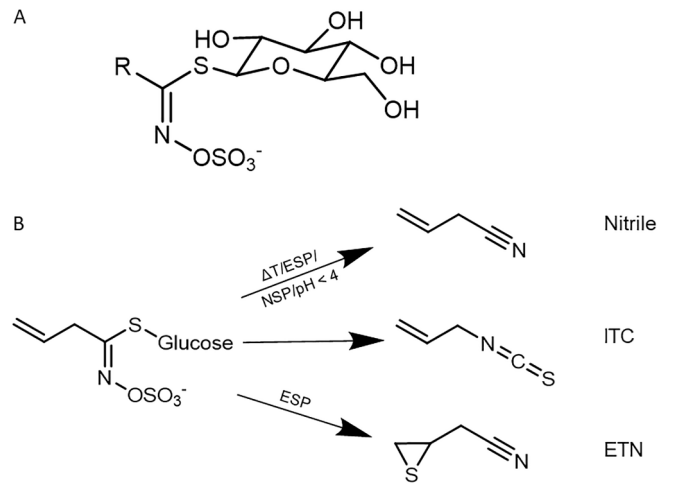


Fig. 1. Core structure of glucosinolates (GLSs) with variable side chain R (A) and products of GLS degradation pathways as shown exemplarily for allyl GLS to form a nitrile, isothiocyanate (ITC) or epithionitrile (ETN) (B). Degradation may happen induced by enzymes and/or thermal treatment. ETNs can only be formed from GLSs with a terminal double bond in their side chain in presence of the epithiospecifier protein (ESP). NSP, nitrile specifier protein.

(Verkerk & Dekker, 2004), but enables thermal degradation of GLSs. Among other factors, thermal GLS stability is dependent on the GLS structure, water content, and differs among different vegetable varieties (Dekker, Hennig, & Verkerk, 2009).

Generally, the sum of all chemical components in food, their organization and molecular interactions have been described as the food matrix. This includes macro- and micronutrients as well as the food's structure in macro- and microscopic scale (Capuano, Oliviero, & van Boekel, 2018). Interactions of food matrix with phytochemicals during food processing have been shown to negatively impact their bioaccessibility (Shahidi & Pan, 2021). Additionally, a distinct influence of the food matrix on the reactivity of food components has been described (Capuano et al., 2018). Within the present study, the term "plant matrix" was regarded as the entirety of water-soluble plant components present in a system apart from GLSs and GLS degradation products.

During thermal treatment, GLSs mainly degrade to their corresponding nitriles (Hanschen, Kühn, Nickel, Rohn, & Dekker, 2018; Williams, Critchley, Pun, Chaliha, & O'Hare, 2009). The stability of individual GLSs during domestic cooking procedures in a variety of different *Brassica* vegetable matrices has been studied and reviewed extensively (Sikorska-Zimny & Beneduce, 2020; Wu et al., 2021). In contrast, studies that follow the fate of the individual GLSs and the formation of their degradation products in plant matrices are scarce. Using plant-free buffered model systems, previously we found nearly equimolar amounts of ITCs during thermal GLS degradation, while the addition of plant matrix increased the rate of GLS breakdown and shifted the rate of formed products heavily towards nitrile formation (Hanschen, Bauer, et al., 2012). Moreover, during boiling experiments with vegetables such as kohlrabi, cabbage, or Brussels sprouts, nitriles where found to be the main GLS degradation products, while only low levels or no ITCs were detected (Ciska, Drabińska, Honke, & Narwojsz, 2015; Hanschen, Kühn, et al., 2018). This strongly suggests that the presence of other components in the plant matrix increases nitrile formation during thermal GLS degradation. Further, the stability of the same GLSs varies between different vegetables, even when grown under the same conditions (Dekker et al., 2009; Hanschen, Kühn, et al., 2018). While the exact mechanism of thermal GLS degradation is still unclear, several studies suggest that, among other factors, the presence of redox-active compounds such as Fe^{2+}-ions or ascorbic acid increases GLS degradation and boosts nitrile formation (Frandsen et al., 2019; Hanschen, Bauer, et al., 2012; Williams et al., 2009).

The aim of the present study was to contribute to the understanding of thermal GLS degradation by investigating the effect of matrix concentration on the thermal degradation pathways of GLSs. In order to obtain model systems with high similarity to actual food systems, differently concentrated hot aqueous extracts ("vegetable broths") of two selected *Brassica* vegetables were prepared. Kohlrabi (*Brassica oleracea* L. var. *gongylodes* cv. 'Kolibri') and red cabbage (*Brassica oleracea* L. var. *capitata* f. *rubra* cv. 'Integro') were chosen as vegetables because they are not only frequently consumed vegetables in Germany, but they also allow for the investigation of different plant organs, namely leaves and stems. Broths were boiled for different periods of time and degradation of GLSs as well as formation of GLS breakdown products were investigated. Additionally, to elucidate the influence of redox-active compounds, the involvement of H_2S was studied. Understanding the mechanisms of thermal GLS degradation in *Brassica* vegetables could help to retain the health-promoting effects of processed *Brassica* foods in the future.

2. Methods

2.1. Chemicals and enzymes

Methylene chloride (GC Ultra Grade), 4-hydroxybenzyl GLS (≥99 %), sinigrin monohydrate (allyl GLS), imidazole (≥99 %), thioacetamide (≥99 %), and lead acetate paper were purchased from Carl Roth GmbH + Co. KG (Karlsruhe, Germany); allyl ITC (≥99 %), aryl sulfatase, (−)-sinigrin hydrate (allyl GLS) (≥99.0 %), benzonitrile (≥99.9 %), 3-butenenitrile (≥98 %), DEAE-Sephadex A-25, 4-pentenenitrile (≥97 %), indol-3-ylacetonitrile (≥98 %), and 3-phenylpropanenitrile (≥99 %) were obtained from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany); 3-butenyl ITC (≥95 %) was purchased from TCI Deutschland GmbH (Eschborn, Germany); $NaSO_4$ anhydrous (≥99 %) was obtained from VWR International GmbH (Darmstadt, Germany); 3-(methylsulfinyl)propyl ITC, 4-(methylthio)butyl ITC (≥98 %), and D,L-goitrin (≥98 %) were purchased from Santa Cruz Biotechnology, Inc. (Heidelberg, Germany); 4-(methylsulfinyl)butyl ITC was purchased from Enzo Life Sciences GmbH (Lörrach, Germany); and 4-(methylthio)butyl GLS (≥99 %) was purchased from Phytolab GmbH & Co. KG (Vestenbergsgreuth, Germany). 1-Cyano-2,3-epithiopropane (≥95 %) was synthetized by Taros Chemicals GmbH Co. KG (Dortmund, Germany); 3-hydroxypent-4-enenitrile (95 %) was purchased from abcr GmbH (Karlsruhe, Germany); and 1-cyano-3,4-epithiobutane was synthetized by ASCA GmbH (Berlin, Germany). 5-(Methylthio) pentanenitrile, 5-(methylsulfinyl)pentanenitrile, and 4-(methylthio) butanenitrile were purchased from SIA Enamine (Riga, Latvia). Methanol (≥99.95 %) and acetonitrile (LC-MS grade) were purchased from Th. Geyer GmbH & Co. KG (Renningen, Germany). Water used in this study was of ultrapure grade.

2.2. Plant material

Kohlrabi (*Brassica oleracea* L. var. *gongylodes* cv. 'Kolibri'; Bejo Samen GmbH, Sonsbeck, Germany) seeds were sown in substrate (Einheitserde P, Einheitserde Werkverband e.V., Germany) in seed growing trays. Five days after germination, plants were transferred into single pots containing soil (Einheitserde T, Einheitserde Werkverband e.V., Germany) and grown in an open-sided greenhouse at the Leibniz Institute of Vegetable and Ornamental Crops (IGZ) in Großbeeren, Germany (52°20′56.0″N 13°18′37.3″E). Water was given as needed and plants were fertilized regularly. After 80 days, kohlrabi plants were harvested and immediately processed further as described below. Kohlrabi plants were grown again the following year under the same conditions and used for follow-up experiments. Additionally, some kohlrabi vegetables were bought at a local supermarket for small additional experiments.

Red cabbage (*Brassica oleracea* L. var. *capitata* f. *rubra* cv. 'Integro'; Bejo Samen GmbH, Sonsbeck, Germany) seeds were sown in substrate

(Einheitserde P). Five days after germination, plants were transferred into single pots filled with soil and grown in an open-sided greenhouse. After 28 days, plants were transferred into an open-air field close to the institute (52°20′58.4″N 13°18′52.9″E), which contains concrete plots of 2 × 2 m filled with loess soil. Each plot contained 16 plants in total with equal distance between all plants. For protection, plants were covered with nets at all times during growth outside. Water was given as needed and plants were fertilized twice following common practice. Plants were harvested after a total of 138 days as fully developed heads and immediately processed further as described below.

2.3. Preparation of vegetable broths

2.3.1. Main experiment

For the main experiment, broths with three different concentrations for both vegetables were produced. Immediately after harvest, kohlrabi stems were randomized into groups of three vegetables each to obtain independently prepared broths. Kohlrabi stems were cut in half, one half of each kohlrabi was peeled after removal of leaves and chopped into small cubes of approx. 0.5 cm length per side and mixed together. For each batch, 150 g of chopped kohlrabi (each mixed from three vegetables) were weighted into a beaker. Beakers were placed on a cooking stove with a watch glass on top and pre-heated for 45 s. Then, for the 1 g/mL broths, 150 mL of boiling water was added; for the 0.5 g/mL broths 300 mL of water and for the 0.25 g/mL broths 600 mL of boiling water was added. Temperature was assessed with a thermometer (Testo 925, Testo SE & Co. KGaA, Titisee-Neustadt, Germany) after addition of boiling water as well as every minute until the temperature reached 90 °C. Then, the heating continued for precisely 10 min. Overall, heating times were between about 20 min for the 1 g/mL broth and about 13 min for the 0.25 g/mL broth. After completion of boiling, broths were filtrated through a paper filter and immediately cooled on ice.

Red cabbage broths were prepared similarly: For each broth, three individual vegetables were used immediately after harvest. Outer leaves and stems were removed and one eighth of each cabbage head was chopped into small pieces of approx. 0.5 cm length per side, and pieces from three cabbages were mixed together. As for kohlrabi, 150 g of pooled cabbage was used to prepare broths with same volumes of water as described above. Heating time were between about 18 min for 1 g/mL broth and about 15 min for 0.25 g/mL broth. For both vegetables, detailed heating times are presented in Supplementary Table S2 and temperature curves are displayed in Supplementary Fig. S1.

After cooling, 10 mL of kohlrabi or red cabbage broths were aliquoted into 20 mL headspace vials and sealed off with an aluminum/PTFE cap. To each kohlrabi aliquot, 75 μL of a 12 mM solution of allyl GLS (sinigrin) in H_2O were added, resulting in a theoretical concentration of 90 μM per vial. Using HPLC-DAD, a concentration of 85 μM was measured. Allyl GLS was added to study GLS stability and formation of degradation products also in an artificially added GLS, which is not naturally present in kohlrabi. Aliquots of broths as well as left-over broth was stored at −60 °C until further analysis.

2.4. Broth for follow-up experiments

After reviewing results from the main experiment, additional broth was prepared to allow for follow-up experiments. Four kohlrabi vegetables were bought at a local supermarket and 1 g/mL broth was prepared from 475 g of chopped vegetables as described above. Freshly prepared broth was stored in 50 mL tubes at − 60 °C until further usage.

2.5. Preparation of a 2 g/mL broth

To investigate the GLS degradation and the formation rate of nitriles in a more concentrated broth, also 2 g/mL broth was prepared. The concentration of 1 g/mL proofed to be the upper limit to achieve by boiling as using less water would not fully cover the vegetable pieces anymore. Therefore, 100 mL of existing 1 g/mL broth (see above) was concentrated using a rotary evaporator (RC 900, KNF DAC GmbH, Hamburg, Germany) at 40 °C and 55 mbar to about 40 mL. The final concentration was established by using a 50 mL volumetric flask. This was done twice independently using the same starting 1 g/mL broth.

2.6. Broth for boiling experiment with thioacetamide

To allow for a larger scale follow-up experiment, kohlrabi was grown again in the following year at IGZ as described above. After 84 days, kohlrabi plants were harvested and directly processed into 2 independent 1 g/mL broths as described before, using 750 g of plant material (from 7 plants each) and 750 mL of water. Broths were stored in 50 mL aliquots at −60 °C until further usage.

2.7. Boiling of differently concentrated vegetable broths

2.7.1. Main experiment

For the main boiling experiment, vials were thawed immediately before analysis. On each day of analysis, 24 aliquots were used, consisting of eight vials for each concentration level for eight different time points (0, 10, 20, 30, 45, 60, 90, and 120 min). Vials were placed into a preheated laboratory shaker (MHR 23, Hettich Benelux B.V., PC Geldermalsen, the Netherlands) and treated for their respective boiling time at 400 rpm and 108 °C. The temperature of the shaker was optimized in preliminary experiments, because it was observed that 100 °C as shaker temperature did not lead to the needed 100 °C inside the vials. After the heat treatment, vials were cooled on ice, uncapped and immediately used for further analysis. For analysis of GLS degradation products, 3 mL of boiled broth were transferred into solvent resistant centrifuge tubes and immediately analyzed as described below. For GLS analysis, 3 mL aliquots of boiled broth were transferred into 5 mL screw cap tubes. After addition of 25 μL of a 1.56 mM solution of 4-hydroxybenzyl GLS (sinalbin) as internal standard (ISTD), samples were stored at −20 °C until final analysis. The experiment was repeated three times on different days using the different broth batches.

2.7.2. Spiking of allyl GLS during boiling

Seven mL of the 1 g/mL kohlrabi broth for follow-up experiments were boiled as described above. For control samples, 83.2 μL of a 12 mM solution of allyl GLS was added before boiling. For treated samples, vials were boiled for 60 min. Then, vials were taken out of the shaker, opened, allyl GLS was added, vials were re-capped and placed back in the shaker. Boiling then continued for 30 or 60 min. Control samples were boiled for 0, 30, 60, 90, and 120 min. Analysis of GLSs and GLS degradation products was done as described above.

2.7.3. Boiling of 2 g/mL broth

The 2 g/mL broth was subsequently boiled as described above. For this experiment, only 7 mL of broth were used. Some previously concentrated broth was also re-diluted to 1 g/mL by using 3.5 mL of 2 g/mL broth and 3.5 mL of water. Again, 75 μL of a 12 mM solution of allyl GLS was added, resulting in a concentration of 128.5 μM. Boiling times were 30, 60, 90, and 120 min for control samples (untreated 1 g/mL broth) and 60 and 120 min for concentrated and reconstituted broth samples. The concentration step, diluting, and subsequent boiling was performed using two replicates made from a single batch of kohlrabi broth. Analysis of GLSs and GLS breakdown products was done as for the main experiment.

2.7.4. Detection of hydrogen sulfide during boiling of kohlrabi broths

Kohlrabi broth with 1 g/mL (prepared as follow-up) was used to test for H_2S formation. Broth samples were boiled as described above, with moisturized lead acetate paper tucked between vial and lid to ensure it is in contact with the gas phase in the vial but not the broth itself during boiling. The experiment was repeated 3 times using the same broth

sample with boiling times of 45 and 90 min.

2.7.5. Boiling of kohlrabi broth with addition of thioacetamide

Kohlrabi broth with 1 g/mL (prepared from self-grown kohlrabi plants in the following year) was used with addition of thioacetamide (TAA) and without (control). Four mL of broth were transferred into vials, and 40 μL of a 12.7 mM solution of allyl GLS were added. For treated samples, 57.2 μL of a 277 mM solution of freshly diluted TAA in water was added, and samples were boiled (conditions see above) for 0, 30, 60, 90, 120, 150, 180, and 240 min. Both control and treatment were done in quadruplet on two days (two repetitions each per day, one per broth per treatment). After boiling, GLSs and GLS breakdown products of all samples were analyzed as described above using 1.5 mL for each analysis.

2.8. Analysis of GLSs as desulfo-GLSs by HPLC-DAD-ToF-MS

For the GLS analysis, the method of Wiesner et al., which is based on the DIN EN ISO 9167-1 protocol, was used with some modifications (Wiesner, Zrenner, Krumbein, Glatt, & Schreiner, 2013). Briefly, 1.5 or 3 mL of boiled broth samples (containing sinalbin as ISTD) were directly loaded on DEAE-Sephadex A-25 ion-exchanger columns and treated with aryl sulfatase overnight. For elution, 1.5 mL of water were used. After filtration through 0.22 μm cellulose acetate filters (Sorenson Bioscience, Salt Lake City, UT, USA), samples were analyzed via HPLC-DAD-ToF-MS as described previously (Hanschen, 2020) with slight modifications: Separation was carried out at 30 °C using a flow rate of 0.4 mL min^{-1}. Eluents were water (A) and acetonitrile (B), starting at 0.2 % B for 2 min, rising to 19.8 % B for 10 min (2 min hold), rising to 50 % B in 1 min (1:50 min hold). The column was washed by rising to 100 % B (3 min hold), followed by decrease to 0.2 % B within 1 min and 5 min post-time. The MS was run in positive polarity using a multimode source and a scan range of 100–1700 *m/z*, further parameters were as described previously (Hanschen, 2020). Identification of desulfo-GLSs and quantification was also done as described previously (Hanschen, 2020).

2.9. Analysis of GLS degradation products by GC-MS

Extraction and analysis of GLS degradation products was carried out as previously reported with small modifications (Wermter, Rohn, & Hanschen, 2020). Benzonitrile (100 μL of 2 mM in methylene chloride) was added to boiled broth aliquots as ISTD. Extraction and GC-MS analysis were performed as described previously (Wermter et al., 2020), but a SGE BPX5 column (30 m × 0.25 mm × 0.25 μm) (Trajan Scientific and Medical, Victoria, Australia) was used, transfer line temperature was set to 250 °C, and the temperature gradient started at 37 °C. Compounds were identified using their retention times and mass spectra by comparing them to authentic standards and using the NIST library. Quantification was carried out using their response factors to the ISTD, which were determined with standards. For tentatively identified compounds, where no standard was commercially available, the response factor of the chemically most similar compound was used.

2.10. Analysis of the redox potential of boiled kohlrabi broths

Redox potential of boiled kohlrabi broths was assessed using a Pt/Ag/AgCl redox electrode (LE501, coupled to a FiveEasy F20, Mettler Toledo, Gießen, Germany). Broth samples (1.5 mL, later used for GLS analysis) were thawed to room temperature, vortexed and measured with the electrode until a stable potential was reached. Each day, the electrode was tested for precision using a 220 mV testing solution (DM Messtechnik, Seeon, Germany).

2.11. Analysis of the total iron content

The total iron content of the *Brassica* vegetable broths was determined by inductively coupled plasma atomic emission spectroscopy (ICP-OES) after microwave pressure digestion as described previously (Hanschen, Kühn, et al., 2018).

2.12. Statistical analysis

To investigate differences between treatments, means were compared using one-way ANOVA followed by Tukey's *post-hoc* test or by Holm-Šidák *post-hoc* test using SigmaPlot version 14.0 (Systat Software Inc., San Jose, CA, USA). In case that test for normality (Shapiro-Wilk) or equal variance (Brown-Forsythe) failed, ANOVA on ranks was performed. Additionally, differences between means were tested by Student's *t*-test using SigmaPlot. A significance level of $p \leq 0.05$ was considered statistically significant. For the correlation of redox potential with nitrile concentration, Pearson correlation was done using SigmaPlot. Experiments were carried out in three independent repetitions unless stated otherwise. Data is shown as means ± standard deviation.

3. Results

After vegetable broth preparation, concentrations of individual GLSs were analyzed. Following the subsequent boiling experiment, degradation of GLSs and accumulation of their respective degradation products was assessed and compared between different matrix concentrations.

3.1. GLS concentration in vegetable broths

Before the subsequent boiling, the concentrations of individual GLSs in kohlrabi and red cabbage broths were assessed (Fig. 2). For both vegetables, concentrations of naturally occurring GLSs were highest in the 1 g/mL broths and declined with declining plant matrix concentration. In broths of both vegetables, the GLS concentration was reduced by about 25 % from 1 g/mL to 0.5 g/mL broths, and by about 50 % from 1 g/mL to 0.25 g/mL broths. In kohlrabi broth (Fig. 2A), the main GLS was 4-(methylthio)butyl GLS (4MTB-GLS) with a concentration of 244 nmol/mL in the 1 g/mL-broth and 110 nmol/mL in the 0.25 g/mL broth, followed by 3-(methylthio)propyl GLS (3MTP-GLS). Additionally, 3-(methylsulfinyl)propyl GLS (3MSOP-GLS), 4-(methylsulfinyl)butyl GLS (4MSOB-GLS), and several indolic GLSs were present in smaller amounts. Allyl GLS was equally concentrated in all broths as it was added artificially to monitor GLS stability and formation of degradation products of an external GLS not naturally present in kohlrabi.

In red cabbage broth (Fig. 2B), 4MSOB-GLS was the most abundant GLS with concentrations ranging between 118 nmol/mL in the 1 g/mL broth and 87 nmol/mL in the 0.25 g/mL broth. Further major GLSs in red cabbage broth were found to be several alkenylic GLSs, while indol-3-ylmethyl GLS (I3M-GLS) was the only abundant indolic GLS. Because starting concentrations in the vegetable broths differed, as a comparable parameter the relative GLS degradation product formation rate was calculated, as explained in section 3.2.

3.2. Degradation of GLSs in kohlrabi broth and formation of their degradation products

For this experiment, aliquots of differently concentrated kohlrabi broth were boiled for up to 120 min, and effects on GLSs as well as on GLS degradation products were analyzed.

Due to the use of differently concentrated broths, GLS concentrations at 0 min (i.e. in freshly prepared broths) differed between broth concentration levels (see Chapter 3.1). As starting concentrations of the corresponding GLSs likely affect absolute amounts of degradation products after boiling, the data of breakdown products was normalized by expressing it as percentage relative to the content of the corresponding GLS at t = 0 min following equation (1):

$$relative\ formation\ of\ degradation\ products\ [\%] = \frac{concentration_{degradation\ product\ at\ t_x}}{concentration_{corresponding\ GLS\ at\ t_0}} \times 100\% \quad (1)$$

In a similar manner, degradation rate of GLSs over time was expressed in percentage relative to the content of the respective GLS at t = 0 min. Therefore, the results are comparable between repetitions and different broths.

During the first 60 min of boiling, only a slow degradation of GLSs for all three matrix concentrations was detected. While allyl GLS stability was the highest of all investigated GLSs with over 90 % remaining after 60 min of boiling, I3M-GLS was the least stable with about 60 % of the initial amounts found after 60 min of boiling for all three matrix concentrations (Fig. 3). At longer boiling times, GLS stability rapidly declined in a concentration dependent manner, showing slower degradation in more diluted broths. Regarding 3MTP-GLS, e.g., recovery of initial GLS amounts dropped from about 80 % after 60 min to only 43.5 % and 58.4 % for 1 g/mL and 0.5 g/mL broths after 90 min, respectively. After 120 min, only 40 % of 3MTP-GLS remained in 1 and 0.5 g/mL broths, while 58.1 % of this GLS were still present in the 0.25 g/mL broth (Fig. 3A). Comparable results were observed for all GLSs investigated in the study (Fig. 3 and Supplementary Fig. S2).

Following the trend of GLS stability, broths of all three concentration levels showed an increase in formation of the corresponding nitriles over time. Generally, nitrile formation rate in 1 g/mL broths was significantly higher compared to 0.25 g/mL broths and 0.5 g/mL broths. For example, after 60 min of boiling, relative degradation of 4MTB-GLS to 5-(methylthio)pentanenitrile (4MTB-CN) was 12.9 % in the 1 g/mL broth, 7.8 % in the 0.5 g/mL broth, and 4.1 % in the 0.25 g/mL broth (Fig. 3B). After another 60 min of boiling, nitrile formation rates reached 55.5 %, 26.3 %, and 8.4 %, resulting in significant differences in formation rates between different matrix concentration levels. Similar results were obtained for all GLSs shown in Fig. 3, with relative formation rates of the corresponding nitriles varying between 35 and 55 % in the 1 g/mL broths. In the 0.25 g/mL broths, generally only low degradation of GLSs to nitriles was observed. Here, the highest nitrile formation rate detected was 13.6 % for 4-(methylthio)butanenitrile (3MTP-CN). The nitrile formation rate in 0.5 g/mL broths was between the results for 1 g/mL broths and 0.25 g/mL broths for all GLSs investigated (Fig. 3 and S2).

As shown for 4-(methylsulfinyl)butanenitrile (3MSOP-CN), presence

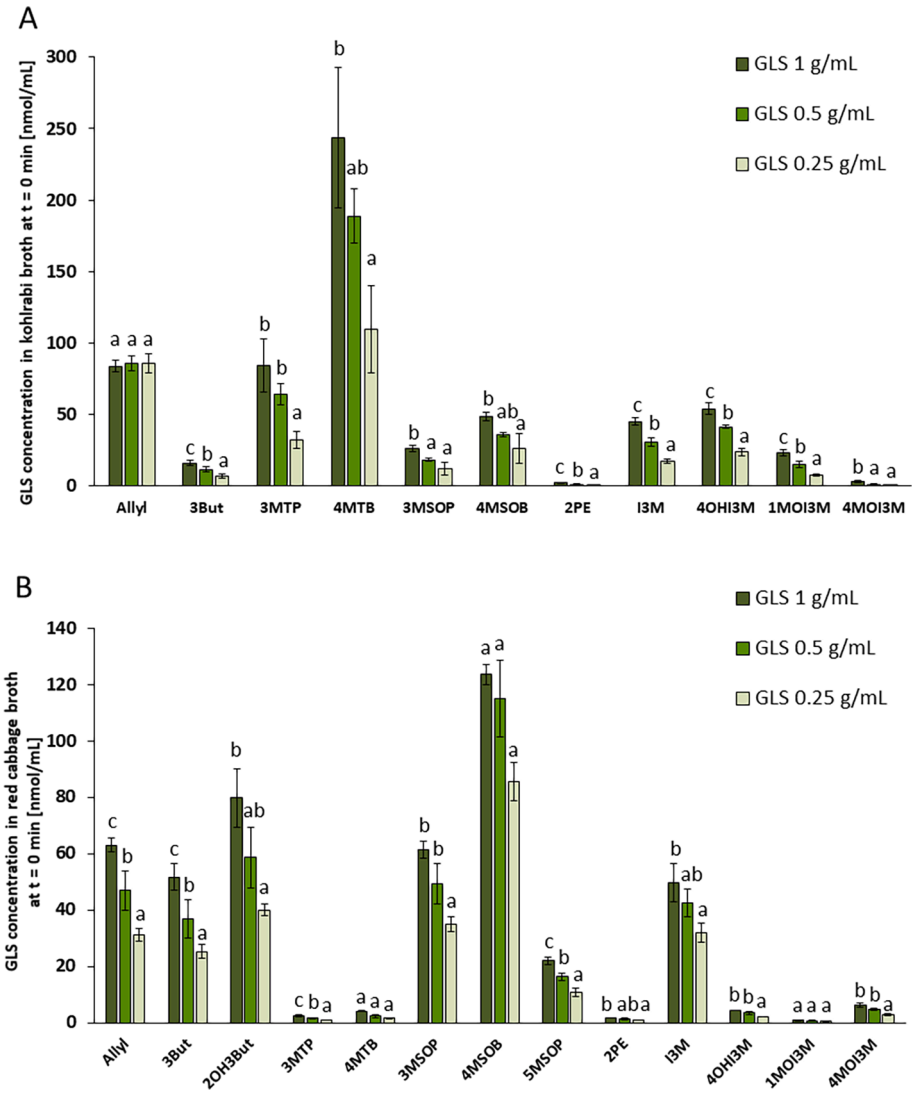


Fig. 2. Glucosinolate (GLS) concentration levels in freshly prepared (A) kohlrabi and (B) red cabbage broths. Abbreviations refer to GLS names as shown in Supplementary Table S1. Values show means ± standard deviation of three independent experiments (n = 3). Significant differences in GLS concentration between differently concentrated broths are indicated with lower case letters from lowest to highest as determined by one-way ANOVA followed by Holm-Šidák *post-hoc* test ($p \leq 0.05$). Allyl GLS was artificially added to kohlrabi broths. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

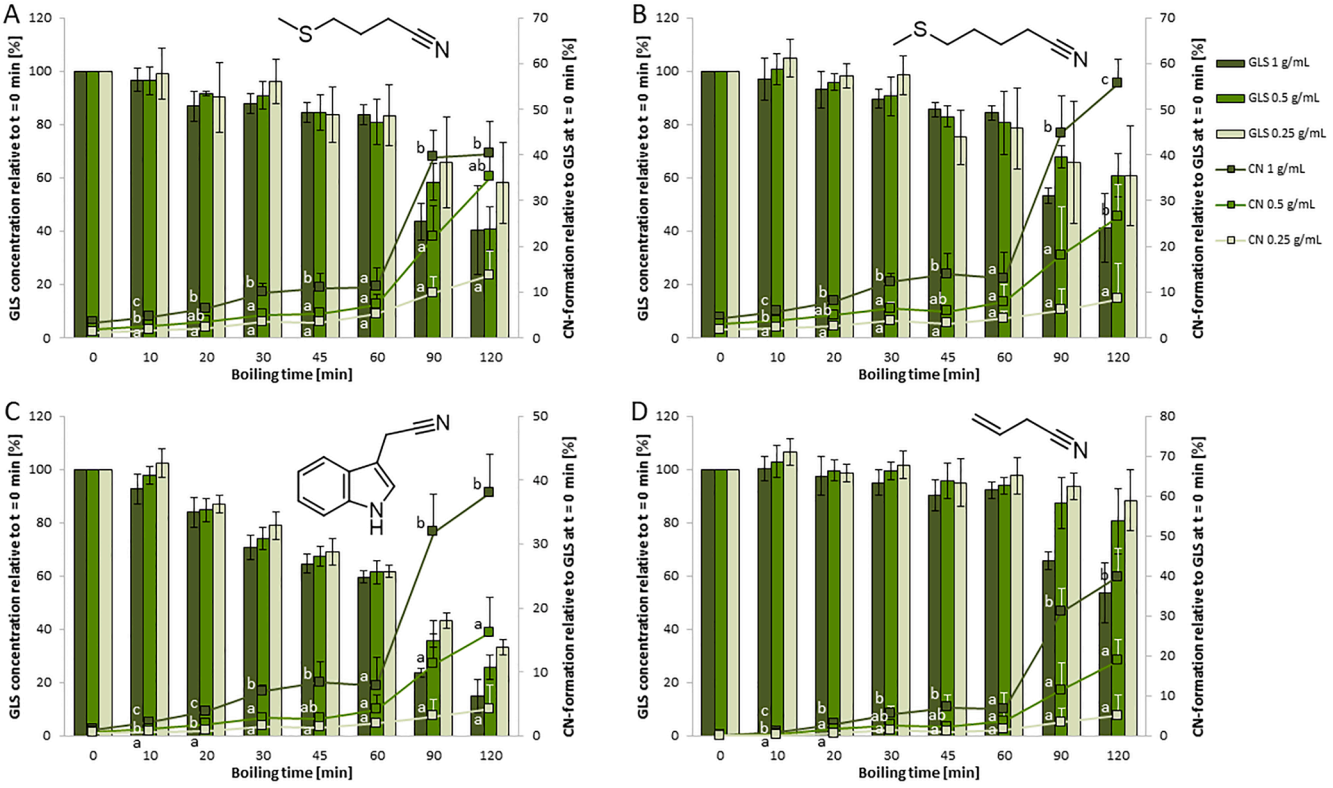


Fig. 3. Effect of different matrix concentrations on glucosinolates (GLSs) and GLS degradation products during boiling of kohlrabi broths: (A) 3-(methylthio)propyl GLS, (B) 4-(methylthio)butyl GLS, (C) indol-3-ylmethyl GLS and (D) artificially added allyl GLS as well as the respective nitriles formed after GLS degradation as indicated by structures. Values show means ± standard deviation of three independent experiments (n = 3). Significant differences in relative formation of GLS degradation products between differently concentrated broths at a given time are indicated with lower case letters from lowest to highest as determined by one-way ANOVA followed by Tukey's *post-hoc* test ($p \leq 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

of a sulfinyl group in the GLS side chain led to comparably low nitrile formation overall, but increasing matrix concentration still markedly increased nitrile formation (Supplementary Fig. S2A). After 120 min of boiling, relative nitrile formation was 4.6 % in the 1 g/mL broth and only 0.25 % in the 0.25 g/mL broth.

Remarkably, the trend of high GLS stability during the first 60 min of boiling, followed by a sharp decrease in GLS stability and increased formation rates of corresponding nitriles in the next 30–60 min of boiling was observed for all GLSs investigated in this study, and it strongly depended on the matrix concentration levels (Fig. 3 and S2). These effects were also observed for allyl GLS, which was added artificially, as well as for its nitrile 3-butenenitrile (Allyl-CN) (Fig. 3D).

Regarding ITCs, they were formed during the initial broth preparation and degraded during subsequent boiling of the broths as shown here for the selected 3MTP-ITC and 4MTB-ITC (Supplementary Fig. S2C and D). The highest ITC concentration (relative to initial GLS levels) was found in 1 g/mL broth at t = 0 min with about 6.5 % for both ITCs shown. During boiling, relative ITC concentration steadily declined so that after 120 min of boiling, it was below 1 % for all broths. No accumulation of ITCs during thermal treatment of kohlrabi broths was observed. These results were similar for further ITCs that were detected in kohlrabi broths, namely allyl ITC and 4MSOB-ITC (not shown, data is available in the Supplementary Table raw data file).

3.3. Degradation of GLSs in red cabbage broth and formation of their degradation products

In contrast to kohlrabi, GLS stability during boiling of red cabbage broths showed no decrease, but rather an increase in GLS recovery after prolonged boiling of red cabbage broths (Fig. 4 and Supplementary Fig. S3). An exception was found for I3M-GLS, where GLS recovery declined to about 50 % for all three matrix concentrations after 120 min of boiling (Fig. 4C). When plotting the relative or absolute peak areas for 4MSOB-GLS and the ISTD over the boiling time, 4MSOB-GLS peak areas did not increase. In contrast, the ISTD areas declined with longer boiling times (Supplementary Fig. S4C and D), which led to an overestimation of GLS levels.

Formation of the corresponding nitriles increased over time during boiling, especially for the 1 g/mL and 0.5 g/mL broths, but less than in kohlrabi. At the same time, accumulation of GLS degradation products in the 0.25 g/mL broths was low over all analyzed products. Relative formation of the nitriles of major GLSs in red cabbage broth, namely Allyl-CN, 5-(methylsulfinyl)pentanenitrile (4MSOB-CN), indole-3-acetonitrile (IAN), and 4-pentenenitrile (3But-CN), peaked at rates between 12.5 % and 20.2 % after 120 min in 1 g/mL broths, which was lower compared to kohlrabi (Fig. 4). Similar to kohlrabi, over all boiling times, relative GLS degradation to the corresponding nitrile was highest in 1 g/mL broths followed by 0.5 g/mL broths and lowest in 0.25 g/mL broths.

Among all investigated nitriles in red cabbage broths, 3-phenylpropanenitrile (2PE-CN) showed the highest relative formation rate from the precursor GLS, reaching 30.3 % after 120 min of boiling in 1 g/mL broth (Supplementary Fig. S3A). However, 2-phenylethyl GLS (2PE-GLS) was only present in minor amounts in the freshly prepared red cabbage broth (Fig. 2B). Additionally, the ETN 1-cyano-2,3-epithiopropane (CETP) was detected in red cabbage broth over all matrix concentrations (Supplementary Fig. S3B). During subsequent boiling, its relative levels steadily declined. Comparable results were observed in further ETNs found in red cabbage broth, namely 1-cyano-3,4-epithiobutane (CETB) and the diastereomeric ETN-isomers formed from 2-OH-3-

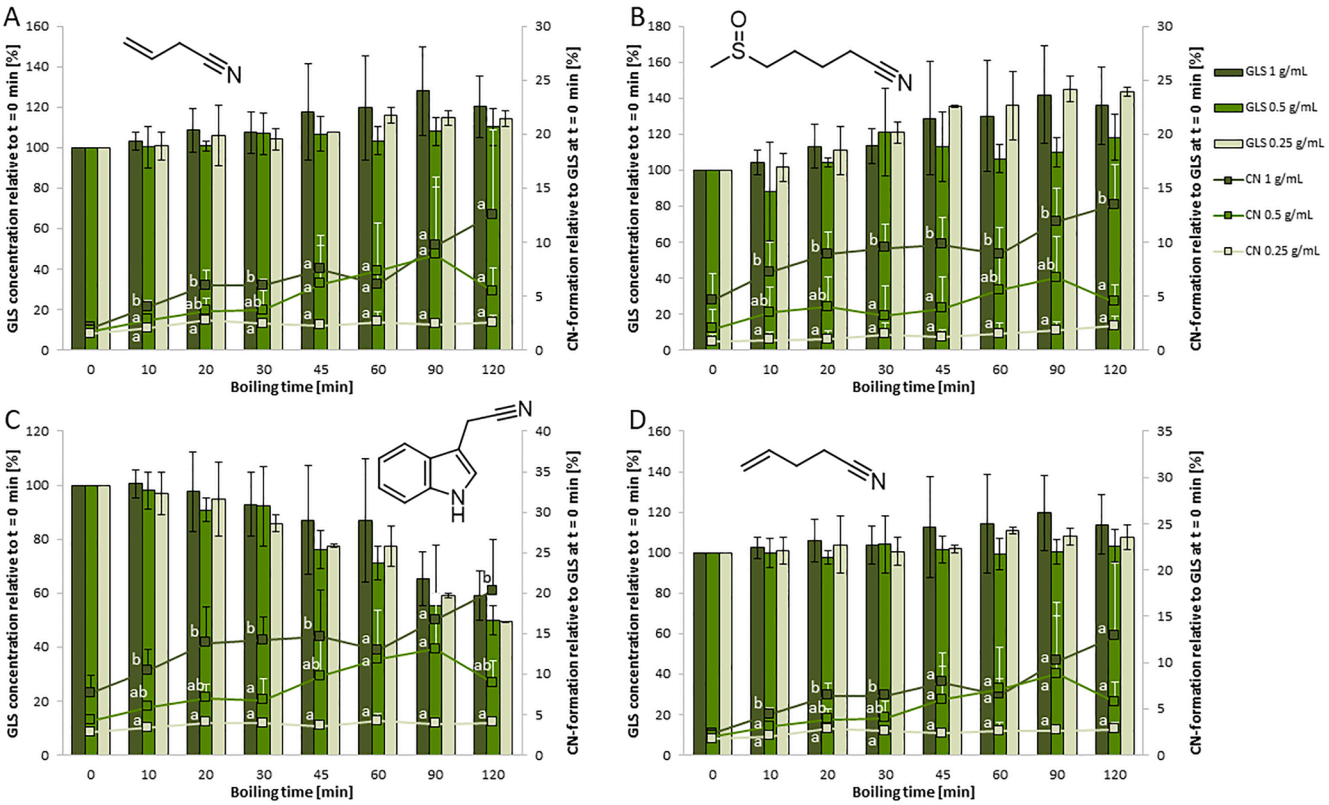


Fig. 4. Effect of different matrix concentrations on glucosinolates (GLSs) and GLS degradation products during boiling of red cabbage broths: (A) allyl GLS, (B) 4-(methylsulfinyl)butyl GLS, (C) indol-3-ylmethyl GLS and (D) 3-butenyl GLS as well as the respective nitriles formed after GLS degradation as indicated by structures. Values show means ± standard deviation of three independent experiments (n = 3). Significant differences in relative formation of GLS degradation products between differently concentrated broths at a given time are indicated with lower case letters from lowest to highest as determined by one-way ANOVA followed by Tukey's *post-hoc* test ($p \leq 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

butenyl GLS (2OH3But-GLS) (not shown, data is available in the Supplementary Table raw data file).

Similarly to kohlrabi broths, ITCs did not accumulate in red cabbage broths during boiling to form considerable amounts, independent from the matrix concentration levels (Supplementary Fig. S3C and D). For allyl ITC, relative formation rates increased during boiling, but values did not exceed 1.5 % at any time for all three matrix concentrations, while 4MSOB-ITC levels declined during boiling. Comparable results were found for all (aryl)aliphatic ITCs of GLSs present in red cabbage broths (not shown, data is available in the Supplementary Table raw data file).

3.4. GLS degradation and formation of degradation products in 2 g/mL kohlrabi broth

For all follow-up experiments only kohlrabi broth was used, because it showed a more pronounced effect during the main boiling experiment. As GLS degradation and formation of nitriles were shown to be concentration dependent, also a 2 g/mL broth was prepared by evaporation of water from freshly produced kohlrabi broth and boiled subsequently. Additionally, broth reconstituted to 1 g/mL was studied. In 2 g/mL broths, relative nitrile formation rate was increased compared to control (untreated 1 g/mL broth) and re-diluted 1 g/mL broths after 60 min (Supplementary Fig. S5). Formation rate of IAN, for example, was 18.4 % (2 g/mL) compared to 0.97 % (control) and 2.8 % (re-diluted 1 g/mL). After an additional 60 min of boiling, breakdown of the GLSs to their nitriles increased. Overall, the highest nitrile formation rate was detected in 2 g/mL broths, where the highest formation rate found was 58.3 % for 4MTB-CN. After 120 min of boiling, nitrile formation was increased in re-diluted broths compared to control samples, but still lower than in 2 g/mL broths.

3.5. Addition of allyl GLS during boiling of kohlrabi broths

A follow-up experiment was set up to investigate the steep increase in GLS degradation and nitrile formation in 1 g/mL kohlrabi broths after 60 min of boiling. Freshly prepared broths were boiled, and after 60 min, vials were briefly opened to add allyl GLS. Unlike in control samples (not opened), in these samples no increase in nitrile formation was detected after an additional 30 or 60 min of boiling (Supplementary Fig. S6).

3.6. Detection of hydrogen sulfide during boiling of kohlrabi broths

A change in the atmosphere during the boiling of broth samples was hypothesized to be a factor responsible for thermal GLS degradation and nitrile formation. Hence, the formation of H_2S during boiling of a 1 g/mL kohlrabi broth was investigated by using moistened lead acetate paper. Already after 45 min of boiling, strong formation of PbS (as black-silver staining) on the paper was visible on the part that was exposed to the atmosphere within the vial during boiling, thereby confirming H_2S formation (Supplementary Fig. S7).

3.7. GLS degradation and formation of degradation products in kohlrabi broth with addition of thioacetamide

As formation of H_2S during boiling of kohlrabi broth was detected, in a further experiment 1 g/mL kohlrabi broth was boiled with the addition of thioacetamide (TAA) to artificially increase the H_2S content in the vial and to study its effect on GLS degradation. In aqueous solution TAA hydrolyses to form H_2S, acetate, and ammonium when heated. Kohlrabi

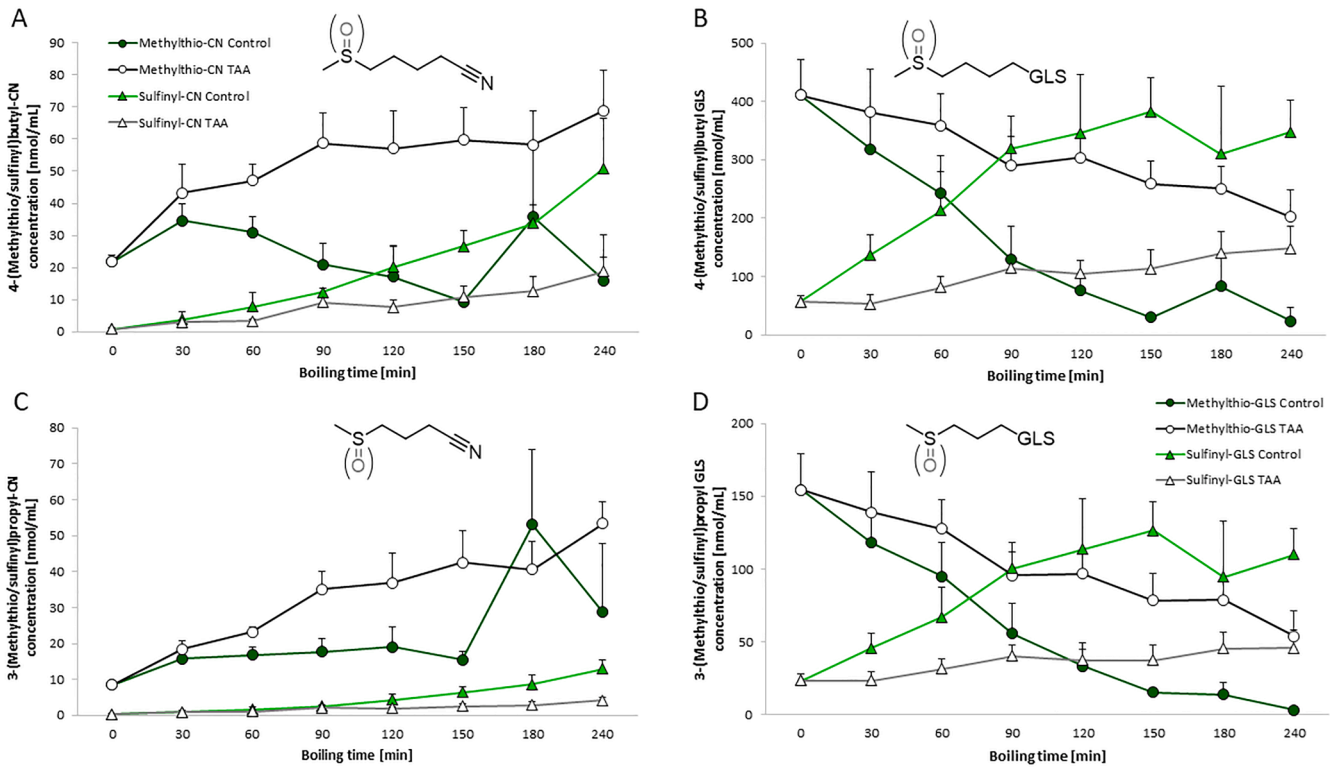


Fig. 5. Influence of thioacetamide (TAA) on glucosinolates (GLSs) and GLS degradation products in kohlrabi broths over time during boiling: (A) 5-(methylthio) pentanenitrile and 5-(methylsulfinyl)pentanenitrile, (B) 4-(methylthio)butyl GLS and 4-(methylsulfinyl)butyl GLS, (C) 4-(methylthio)butanenitrile and 4-(methylsulfinyl)butanenitrile, and (D) 3-(methylthio)propyl GLS and 3-(methylsulfinyl)propyl GLS as indicated by structures. Values show means ± standard deviation of two repetitions of two independent experiments each (n = 4). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

broths were boiled for up to 240 min to monitor GLSs and GLS breakdown products over a longer period of time (Fig. 5, additional results available in Supplementary Fig. S8), and different concentrations between treatments were tested for significance using Student's *t*-test ($p \leq 0.05$). In control samples, 4MTB-GLS and 3MTP-GLS both rapidly degraded during boiling. In contrast to earlier results (main experiment), only low amounts of nitriles were formed. Instead, both GLSs were oxidized to their sulfinyl analogues (Fig. 5). At the same time, the concentrations of the respective sulfinyl nitriles, namely 4MSOB-CN and 3MSOP-CN, increased over time. Addition of TAA slowed the oxidation process for both 4MTB-GLS and 3MTP-GLS (Fig. 5B and 5D), resulting in significantly higher concentrations of these GLSs after 60 min and 90 min of boiling, respectively, and higher concentrations of their corresponding nitriles compared to control after 60 min of boiling (with the exception of t = 180 min) (Fig. 5A and 5C). Simultaneously, significantly lower concentrations of the respective sulfinyl GLSs and the corresponding sulfinyl nitriles (significant after 90 min and 120 min for 4MSOB-CN and 3MSOP-CN, respectively) were detected. For example, 4MTB-GLS concentration was 23.5 nmol/mL in control and 202 nmol/mL in TAA samples while 4MSOB-GLS concentration was 348 nmol/mL in control and 148 nmol/mL in TAA samples after 240 min of boiling (Fig. 5B). Further evidence confirming the oxidation process is that the respective sum of both GLSs for 4MTB/4MSOB-GLS and 3MTP/3MSOP-GLS showed no difference between control and TAA treatment at all boiling times (Supplementary Fig. S8C and D). For the externally added allyl GLS, formation of Allyl-CN in samples containing TAA exceeded control samples between 30 and 150 min of boiling, while no difference in GLS stability between both treatments was found (Supplementary Figs. S8A and B).

3.8. Analysis of the redox potential of boiled kohlrabi broths

Formation of H_2S during boiling of kohlrabi broths was demonstrated and excess H_2S formation was shown to influence both GLS stability and redox reactions during thermal degradation. Therefore, redox potential of boiled kohlrabi broth samples with and without TAA addition was analyzed and correlated with individual nitrile formation rates using Pearson correlation. Results are shown for the four most abundant nitriles, namely 4MTB-CN, 3MTP-CN, Allyl-CN, and 4MSOB-CN in Fig. 6. With the exception of 4MSOB-CN without TAA, all correlations of redox potential with nitrile concentrations were significant ($p < 0.05$), with five of the eight correlations being highly significant ($p < 0.01$).

4. Discussion

Thermal degradation of GLSs was hypothesized to be influenced not only by heat itself, but also by the presence of plant matrix components. Here, this effect was assessed using differently concentrated vegetable broth samples from kohlrabi and red cabbage. Moreover, additional follow-up experiments were conducted to elucidate the mechanisms behind plant matrix interactions with GLSs during thermal treatment.

Overall, the GLS profiles of vegetables and broths mirrored that of previous studies, with kohlrabi being rich in methylthioalkyl GLSs and red cabbage being rich in the methylsulfinylalkyl GLS 4MSOB-GLS as well as further methylthioalkyl GLSs and alkenyl-GLSs (Ciska, Martyniak-Przybyszewska, & Kozlowska, 2000; Park et al., 2012). Here, the relative GLS concentrations (as an indication of leaching rate) in freshly prepared broths was slightly higher (1/0.75/0.5) as would have been expected by equal distribution of GLSs between vegetable and water during boiling (1/0.67/0.4). Likely, the increasing amounts of water lead to higher extractability of GLSs, caused by a higher

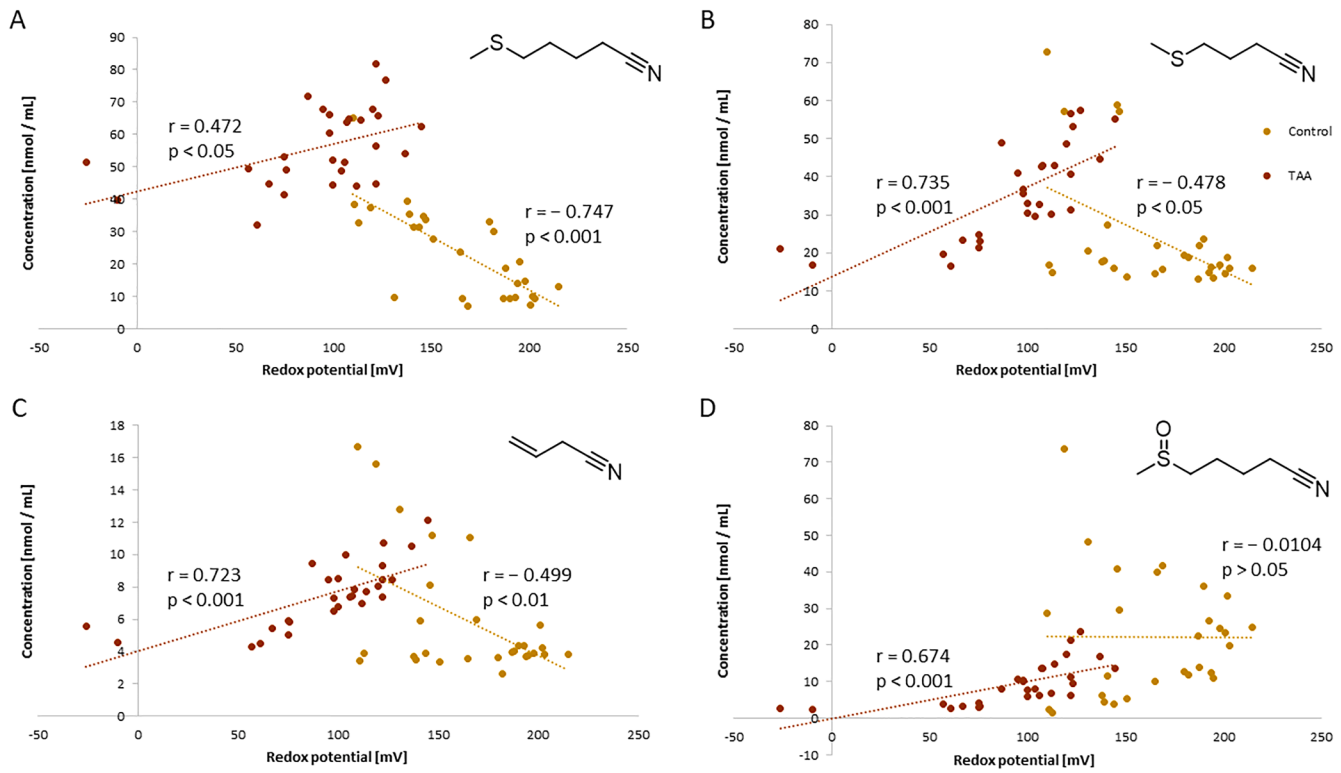


Fig. 6. Correlations of individual nitrile concentrations in boiled kohlrabi broths with and without addition of thioacetamide (TAA) with the redox potential of the broths: (A) 5-(methylthio)pentanenitrile, (B) 4-(methylthio)butanenitrile, (C) 3-butenenitrile and (D) 5-(methylsulfinyl)pentanenitrile as indicated by structures. Correlations were tested for significance by using Pearson correlation, and correlation coefficients and *p*-values are given. Values were produced from two independent experiments with two repetitions each (n = 4). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

concentration difference. This is supported by the leaching ratios of total GLSs ranging between 31 % and 82 % (Supplementary Fig. S9). Similar leaching rates were reported previously: after 15 min of boiling of vegetables in a 1:1 ratio with water (i.e.1 g/mL), 50 % of 4MTB-GLS leached out of kohlrabi and 40 % of 3But-GLS leached out of red cabbage (Hanschen, Kühn, et al., 2018).

In the freshly prepared broths (t = 0 min), next to GLSs especially in the 1 g/mL broths also low amounts of GLS degradation products were detected (Figs. 3 and 4, Supplementary Fig. S2 and S3). Likely, the initial levels of ITCs and ETNs were mainly based on enzymatic hydrolysis of GLSs during chopping of vegetables and during the early phase of broth preparation as myrosinase was still active until reaching temperatures above 60 °C (Hanschen, Kühn, et al., 2018). Due to the higher plant ratio, concentrated broth had lower starting temperatures during broth preparation (1 g/mL: 55–62 °C, see Supplementary Fig. S1) compared to the less concentrated ones, thereby allowing for more enzymatic hydrolysis of GLSs to ITCs and ETNs before enzyme inactivation. During the subsequent boiling, only thermal degradation of GLS is expected to have taken place which mainly yields nitriles in vegetables (Hanschen, Kühn, et al., 2018). Here, ITC levels (formed previously during broth preparation) usually declined, likely due to thermal degradation. ITCs are prone to nucleophilic attack and the half-life of 4MSOB-ITC in broccoli extract was reported to be 1.5 h at pH 6 and 90 °C (Y. Wu, Mao, You, & Liu, 2014). Interestingly, allyl ITC from red cabbage showed a different trend. Although only low amounts were found in all three broth concentrations at t = 0 min, ITC levels slightly increased during boiling (Supplementary Fig. S3C). Therefore, it is suspected that its thermal formation rates exceeded degradation. ETNs did not accumulate during the subsequent boiling, but were also degraded over time. Especially CETP was previously shown to be very instable under aqueous heating and 2-aminothiophene was shown to be its main product (Hanschen, Kaufmann, et al., 2018).

During boiling of differently concentrated *Brassica* vegetable broths, GLSs were thermally degraded. Generally, stability of aliphatic GLSs during boiling was high for the first 60 min, while indolic GLSs showed considerable continuous degradation during the boiling process (Figs. 3 and 4, Supplementary Fig. S2 and S3). Similarly, in previous studies indolic GLSs were found to be more labile compared to aliphatic GLSs (Hanschen et al., 2012; Oerlemans, Barrett, Suades, Verkerk, & Dekker, 2006; Volden, Wicklund, Verkerk, & Dekker, 2008). In red cabbage broths, most GLSs showed high stability and levels seemingly even increased with prolonged boiling. However, the relative peak areas of 4MSOB-GLS and the absolute peak areas of the ISTD revealed that 4MSOB-GLS levels indeed slightly decreased during boiling, but ISTD peak areas simultaneously decreased to a much greater extend, causing an over-quantification of 4MSOB-GLS during later boiling times (Supplementary Fig. S4C and D). In kohlrabi broth samples, this effect was not observed (Supplementary Fig. S4A and B). As the broths were used for GLS analysis without further purification, alteration of matrix components might have negatively impacted ISTD binding to the Sephadex-columns during analysis, leading to overestimation of the GLSs, thereby even compensating for GLS degradation during boiling. This should be considered for similar experiments in the future.

Regarding these differences in GLS stability between red cabbage and kohlrabi broth, it is important to consider that different plant organs were used for broth preparation. Indeed, at similar matrix concentration levels, GLS leaching levels from red cabbage were lower than from kohlrabi (Supplementary Fig. S9). Moreover, the vegetables likely differed in their phytochemical profile, which will affect the composition of the broth. Likely, the broth matrix contained all kinds of water-soluble plant components that may have affected thermal stability of GLS, such as amino acids, carbohydrates, further phytochemicals such as polyphenols and vitamins, as well as inorganic salts. In red cabbage broths, likely also anthocyanins were present. Correlating the presence

of specific matrix components with GLS stability could help to understand the mechanisms of thermal degradation of GLSs in the future. Vegetable broths are usually prepared from more than just one vegetable, making the matrix composition even more complex. Interestingly, a previous study found that heating of broccoli together with onions significantly increased 4MSOB-GLS stability (Giambanelli et al., 2015). The study did not follow up on GLS degradation products leaving room for further investigations of binary or even more complex food systems. Water content was also shown to affect thermal GLS stability: in a modelling study, Oliviero et al. monitored GLS degradation in freeze dried, inactivated broccoli powder, to which they added different amounts of water (Oliviero, Verkerk, & Dekker, 2012). For temperatures up to 100 °C, they found increased stability of GLSs to be correlated with low water content, whereas at 120 °C, it was the opposite. Therefore, the complex interaction of GLSs with plant matrix needs further investigation.

In the present study, after 60 min of boiling of kohlrabi broth, GLS degradation strongly increased as did the formation of the corresponding nitriles (Fig. 3 and S2). This effect increased with increasing matrix concentrations, as relative nitrile formation was highest in 1 g/mL broths for all GLSs investigated. Even more, when increasing matrix concentration to 2 g/mL, nitrile formation rate was further increased (Supplementary Fig. S5).

Similar to results in kohlrabi broths, for all investigated GLSs in red cabbage broths, increased nitrile formation over time and highest nitrile formation rates in 1 g/mL broths were observed (Fig. 4 and S3). Therefore, vegetable matrix has a pronounced, concentration-dependent influence on the thermal degradation of GLSs to form nitriles, while during boiling, ITC formation seems to play an insignificant role. As ferrous ions were previously reported to affect thermal GLS stability, total iron content of vegetable broths was analyzed and ranged between 0.003 and 0.012 mM (Supplementary Fig. S10). While iron concentration trends generally followed the overall matrix concentrations, iron levels were low and also did not explain the sharp increase in nitrile formation in kohlrabi broths. Hence, in the present study ferrous ions most likely were only a minor factor directly affecting thermal GLS stability.

We hypothesized that additional factors might influence GLS reactivity, which need to be formed during boiling before activating GLS breakdown. Therefore, a follow-up experiment was conducted where vials were opened after 60 min of boiling to add allyl GLS, recapped, and boiling continued for 30 or 60 min (Supplementary Fig. S6). Surprisingly, when opening vials mid-boiling, the ability to form nitriles was lost completely, so we hypothesized that the gas phase inside the vial affected GLS stability. Moreover, previous work showed that various sulfur compounds can be formed from *Brassica* vegetable matrix (Chin & Lindsay, 1993). Formation of H_2S during boiling of 1 g/mL kohlrabi broth was subsequently proven using lead acetate paper (Supplementary Fig. S7). As H_2S (i.e. S^{2-}) is the most reduced form of sulfur, we hypothesized that a) redox reactions influence GLS stability and formation of different degradation products, b) a reductive atmosphere is formed inside the vials during boiling, and c) opening the vials mid-boiling disturbs this atmosphere enough to stop further nitrile formation.

To verify, excess H_2S was added to a follow-up 1 g/mL kohlrabi broth by adding TAA, and redox potentials of boiled broths were assessed. In contrast to earlier results, control samples showed only moderate nitrile formation and no increasing trend during boiling, but instead pronounced oxidation of methylthioalkyl GLSs to methylsulfinylalkyl GLSs (Fig. 5 and S8), the latter showing higher thermal stability. Methylthioalkyl GLSs such as 4MTB-GLS were previously identified to be more susceptible to heat compared to the sulfinyl analogs (Hanschen, Platz, et al., 2012), and this was also observed in the main boiling experiment (see Fig. 3A and S2A). As broths used for this experiment were produced from plants grown in a different year, likely their phytochemical composition varied tremendously, as it was shown previously (Aires et al., 2011; Ciska et al., 2000), which could explain the different response of GLSs to heating. The oxidation of 4MTB-GLS to 4MSOB-GLS was demonstrated before, but so far only using oxidative agents like peroxides in buffer systems, but not during boiling of *Brassica* foods (Barillari et al., 2005; Iori, Bernardi, Gueyrard, Rollin, & Palmieri, 1999). Interestingly, addition of TAA prevented oxidation of the sulfur atom in the GLS side chain and led to increased nitrile formation similar to the main experiment (Fig. 5).

Plotting redox potentials of boiled broth samples against analyzed nitrile concentrations showed strong correlations, which were even more pronounced in samples containing TAA, possibly because the redox state was stabilized by addition of excess additive (Fig. 6). Strikingly, slopes of linear regression were reversed when comparing samples with and without TAA addition. Therefore, thermal degradation of GLSs to nitriles is strongly affected by the matrix's redox state.

However, as also Allyl-CN formation increased due to presence of TAA between 30 and 150 min of boiling (Supplementary Fig. S8A), it is likely that H_2S also directly affects nitrile release during thermal degradation of GLSs. Uda et al. reported that in presence of ferrous ions, thiols can block the Lossen-like rearrangement of the GLS-aglycon during enzymatic degradation by accelerating the desulfuration, thereby increasing nitrile release (Uda, Kurata, & Arakawa, 1986). Similar mechanisms might also play a role in the effect of thiols on thermal decomposition of GLSs to nitriles.

Further research is needed to understand the mechanisms of interaction between GLSs and plant matrix components, which is essential to increase thermal GLS stability and therefore retain the health-promoting effects of *Brassica* vegetables after cooking procedures.

5. Conclusion

In the present study, *Brassica* vegetable matrix was shown to increase nitrile formation from GLSs during boiling in a concentration-dependent manner. The redox potential of *Brassica* vegetable broths seems to be a key factor influencing GLS stability during thermal treatment, which leads to nitrile formation after GLS degradation. As it was shown based on the example of H_2S, redox-active agents are capable of manipulating the redox potential of vegetable systems, influencing GLS degradation. Therefore, differences in redox-active compounds in different vegetables may also account for different responses of GLSs to thermal processing. Determination of further mechanisms that affect redox potentials of plant matrices and expansion from model experiments using broths to complete vegetable systems will help to better understand thermal GLS breakdown in the future. This will allow to retain the health-promoting effects of *Brassica* vegetables in human nutrition.

CRediT authorship contribution statement

Matthias Renz: Conceptualization, Methodology, Investigation, Visualization, Writing – original draft. **Matthijs Dekker:** Conceptualization, Methodology, Writing – review & editing. **Sascha Rohn:** Writing – review & editing, Supervision. **Franziska S. Hanschen:** Conceptualization, Methodology, Writing – review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

The study is part of the project OPTIGLUP that is funded by the Leibniz Association (Leibniz-Junior Research Group; J16/2017). Bejo Samen GmbH is gratefully thanked for providing the kohlrabi and red cabbage seeds. Elke Büsch is gratefully thanked for raising the plants and Jessica Eichhorn for excellent technical assistance.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2022.134594.

References

Aires, A., Fernandes, C., Carvalho, R., Bennett, R. N., Saavedra, M. J., & Rosa, E. A. S. (2011). Seasonal effects on bioactive compounds and antioxidant capacity of six economically important brassica vegetables. *Molecules, 16*(8), 6816–6832. https://doi.org/10.3390/molecules16086816

Barillari, J., Canistro, D., Paolini, M., Ferroni, F., Pedulli, G. F., Iori, R., & Valgimigli, L. (2005). Direct antioxidant activity of purified glucoerucin, the dietary secondary metabolite contained in rocket (Eruca sativa Mill.) seeds and sprouts. *Journal of Agricultural and Food Chemistry, 53*(7), 2475–2482. https://doi.org/10.1021/jf047945a

Basten, G. P., Bao, Y., & Williamson, G. (2002). Sulforaphane and its glutathione conjugate but not sulforaphane nitrile induce UDP-glucuronosyl transferase (UGT1A1) and glutathione transferase (GSTA1) in cultured cells. *Carcinogenesis, 23*(8), 1399–1404. https://doi.org/10.1093/carcin/23.8.1399

Blazevic, I., Montaut, S., Burcul, F., Olsen, C. E., Burow, M., Rollin, P., & Agerbirk, N. (2020). Glucosinolate structural diversity, identification, chemical synthesis and metabolism in plants. *Phytochemistry, 169*, Article 112100. https://doi.org/10.1016/j.phytochem.2019.112100

Burow, M., & Wittstock, U. (2009). Regulation and function of specifier proteins in plants. *Phytochemistry Reviews, 8*(1), 87–99. https://doi.org/10.1007/s11101-008-9113-5

Capuano, E., Oliviero, T., & van Boekel, M. A. J. S. (2018). Modeling food matrix effects on chemical reactivity: Challenges and perspectives. *Critical Reviews in Food Science and Nutrition, 58*(16), 2814–2828. https://doi.org/10.1080/10408398.2017.1342595

Chin, H.-W., & Lindsay, R. C. (1993). Volatile sulfur compounds formed in disrupted tissues of different cabbage cultivars. *Journal of Food Science, 58*(4), 835–839. https://doi.org/10.1111/j.1365-2621.1993.tb09370.x

Ciska, E., Drabińska, N., Honke, J., & Narwojsz, A. (2015). Boiled Brussels sprouts: A rich source of glucosinolates and the corresponding nitriles. *Journal of Functional Foods, 19*, 91–99. https://doi.org/10.1016/j.jff.2015.09.008

Ciska, E., Martyniak-Przybyszewska, B., & Kozlowska, H. (2000). Content of glucosinolates in cruciferous vegetables grown at the same site for two years under different climatic conditions. *Journal of Agricultural and Food Chemistry, 48*(7), 2862–2867. https://doi.org/10.1021/jf981373a

Dejanovic, G. M., Asllanaj, E., Gamba, M., Raguindin, P. F., Itodo, O. A., Minder, B., … Kern, H. (2021). Phytochemical characterization of turnip greens (Brassica rapa ssp. rapa): A systematic review. *PLoS One, 16*(2), e0247032. https://doi.org/10.1371/journal.pone.0247032

Dekker, M., Hennig, K., & Verkerk, R. (2009). Differences in thermal stability of glucosinolates in five brassica vegetables. *Czech Journal of Food Sciences, 27*, S85–S88. https://doi.org/10.17221/1079-CJFS

Frandsen, H. B., Sorensen, J. C., Jensen, S. K., Markedal, K. E., Joehnke, M. S., Maribo, H., … Sorensen, H. (2019). Non-enzymatic transformations of dietary 2-hydroxyalkenyl and aromatic glucosinolates in the stomach of monogastrics. *Food Chemistry, 291*, 77–86. https://doi.org/10.1016/j.foodchem.2019.03.136

Giambanelli, E., Verkerk, R., Fogliano, V., Capuano, E., D'Antuono, L. F., & Oliviero, T. (2015). Broccoli glucosinolate degradation is reduced performing thermal treatment in binary systems with other food ingredients. *RSC Advances, 5*(82), 66894–66900. https://doi.org/10.1039/C5RA11409H

Giorgetti, L., Giorgi, G., Cherubini, E., Gervasi, P. G., Della Croce, C. M., Longo, V., & Bellani, L. (2018). Screening and identification of major phytochemical compounds in seeds, sprouts and leaves of Tuscan black kale Brassica oleracea (L.) ssp acephala (DC) var. sabellica L. *Natural Product Research, 32*(14), 1617–1626. https://doi.org/10.1080/14786419.2017.1392953

Hanschen, F. S. (2020). Domestic boiling and salad preparation habits affect glucosinolate degradation in red cabbage (Brassica oleracea var. capitata f. rubra). *Food Chemistry, 321*, Article 126694. https://doi.org/10.1016/j.foodchem.2020.126694

Hanschen, F. S., Bauer, A., Mewis, I., Keil, C., Schreiner, M., Rohn, S., & Kroh, L. W. (2012). Thermally induced degradation of aliphatic glucosinolates: Identification of intermediary breakdown products and proposed degradation pathways. *Journal of Agricultural and Food Chemistry, 60*(39), 9890–9899. https://doi.org/10.1021/jf302744y

Hanschen, F. S., Kaufmann, M., Kupke, F., Hackl, T., Kroh, L. W., Rohn, S., & Schreiner, M. (2018). Brassica vegetables as sources of epithionitriles: Novel secondary products formed during cooking. *Food Chemistry, 245*, 564–569. https://doi.org/10.1016/j.foodchem.2017.10.124

Hanschen, F. S., Kühn, C., Nickel, M., Rohn, S., & Dekker, M. (2018). Leaching and degradation kinetics of glucosinolates during boiling of Brassica oleracea vegetables and the formation of their breakdown products. *Food Chemistry, 263*, 240–250. https://doi.org/10.1016/j.foodchem.2018.04.069

Hanschen, F. S., Platz, S., Mewis, I., Schreiner, M., Rohn, S., & Kroh, L. W. (2012). Thermally induced degradation of sulfur-containing aliphatic glucosinolates in broccoli sprouts (Brassica oleracea var. italica) and model systems. *Journal of Agricultural and Food Chemistry, 60*(9), 2231–2241. https://doi.org/10.1021/jf204830p

Iori, R., Bernardi, R., Gueyrard, D., Rollin, P., & Palmieri, S. (1999). Formation of glucoraphanin by chemoselective oxidation of natural glucoerucin: A chemoenzymatic route to sulforaphane. *Bioorganic & Medicinal Chemistry Letters, 9*(7), 1047–1048. https://doi.org/10.1016/S0960-894X(99)00136-5

Kupke, F., Herz, C., Hanschen, F. S., Platz, S., Odongo, G. A., Helmig, S., … Lamy, E. (2016). Cytotoxic and genotoxic potential of food-borne nitriles in a liver in vitro model. *Scientific Reports, 6*, 37631. https://doi.org/10.1038/srep37631

Mitsiogianni, M., Koutsidis, G., Mavroudis, N., Trafalis, D. T., Botaitis, S., Franco, R., … Panayiotidis, M. I. (2019). The role of isothiocyanates as cancer chemo-preventive, chemo-therapeutic and anti-melanoma agents. *Antioxidants, 8*(4). https://doi.org/10.3390/antiox8040106

Oerlemans, K., Barrett, D. M., Suades, C. B., Verkerk, R., & Dekker, M. (2006). Thermal degradation of glucosinolates in red cabbage. *Food Chemistry, 95*(1), 19–29. https://doi.org/10.1016/j.foodchem.2004.12.013

Oliviero, T., Verkerk, R., & Dekker, M. (2012). Effect of water content and temperature on glucosinolate degradation kinetics in broccoli (Brassica oleracea var. italica). *Food Chemistry, 132*(4), 2037–2045. https://doi.org/10.1016/j.foodchem.2011.12.045

Park, W. T., Kim, J. K., Park, S., Lee, S.-W., Li, X., Kim, Y. B., … Park, S. U. (2012). Metabolic profiling of glucosinolates, anthocyanins, carotenoids, and other secondary metabolites in kohlrabi (Brassica oleracea var. gongylodes). *Journal of Agricultural and Food Chemistry, 60*(33), 8111–8116. https://doi.org/10.1021/jf301667j

Shahidi, F., & Pan, Y. (2021). Influence of food matrix and food processing on the chemical interaction and bioaccessibility of dietary phytochemicals: A review. *Critical Reviews in Food Science and Nutrition, 1–25*. https://doi.org/10.1080/10408398.2021.1901650

Sikorska-Zimny, K., & Beneduce, L. (2020). The glucosinolates and their bioactive derivatives in Brassica: A review on classification, biosynthesis and content in plant tissues, fate during and after processing, effect on the human organism and interaction with the gut microbiota. *Critical Reviews in Food Science and Nutrition, 1–28*. https://doi.org/10.1080/10408398.2020.1780193

Uda, Y., Kurata, T., & Arakawa, N. (1986). Effects of thiol compounds on the formation of nitriles from glucosinolates. *Agricultural and Biological Chemistry, 50*(11), 2741–2746. https://doi.org/10.1080/00021369.1986.10867833

Veeranki, O. L., Bhattacharya, A., Tang, L., Marshall, J. R., & Zhang, Y. (2015). Cruciferous vegetables, isothiocyanates, and prevention of bladder cancer. *Current Pharmacology Reports, 1*(4), 272–282. https://doi.org/10.1007/s40495-015-0024-z

Verkerk, R., & Dekker, M. (2004). Glucosinolates and myrosinase activity in red cabbage (Brassica oleracea L Var. Capitata f. rubra DC.) after Various Microwave Treatments. *Journal of Agricultural and Food Chemistry, 52*(24), 7318–7323. https://doi.org/10.1021/jf0493268

Volden, J., Wicklund, T., Verkerk, R., & Dekker, M. (2008). Kinetics of changes in glucosinolate concentrations during long-term cooking of white cabbage (Brassica oleracea L. ssp. capitata f. alba). *Journal of Agricultural and Food Chemistry, 56*(6), 2068–2073. https://doi.org/10.1021/jf0731999

Wermter, N. S., Rohn, S., & Hanschen, F. S. (2020). Seasonal Variation of Glucosinolate Hydrolysis Products in Commercial White and Red Cabbages (Brassica oleracea var. capitata). *Foods, 9*(11), 1682. https://doi.org/10.3390/foods9111682

Wiesner, M., Zrenner, R., Krumbein, A., Glatt, H., & Schreiner, M. (2013). Genotypic variation of the glucosinolate profile in pak choi (Brassica rapa ssp. chinensis). *Journal of Agricultural and Food Chemistry, 61*(8), 1943–1953. https://doi.org/10.1021/jf303970k

Williams, D. J., Critchley, C., Pun, S., Chaliha, M., & O'Hare, T. J. (2009). Differing mechanisms of simple nitrile formation on glucosinolate degradation in Lepidium sativum and Nasturtium officinale seeds. *Phytochemistry, 70*(11), 1401–1409. https://doi.org/10.1016/j.phytochem.2009.07.035

Wittstock, U., & Burow, M. (2010). Glucosinolate breakdown in Arabidopsis: Mechanism, regulation and biological significance. *The arabidopsis book, 8*, e0134–e. https://doi.org/10.1199/tab.0134

Witzel, K., Abu Risha, M., Albers, P., Börnke, F., & Hanschen, F. S. (2019). Identification and characterization of three epithiospecifier protein isoforms in *Brassica oleracea*. *Frontiers in Plant Science, 10*, 1552–1552. https://doi.org/10.3389/fpls.2019.01552.

Wu, X., Huang, H., Childs, H., Wu, Y., Yu, L., & Pehrsson, P. R. (2021). Glucosinolates in brassica vegetables: Characterization and factors that influence distribution, content, and Intake. *Annual Review of Food Science and Technology*. https://doi.org/10.1146/annurev-food-070620-025744

Wu, Y., Mao, J., You, Y., & Liu, S. (2014). Study on degradation kinetics of sulforaphane in broccoli extract. *Food Chemistry, 155*, 235–239. https://doi.org/10.1016/j.foodchem.2014.01.042