JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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Thermally Induced Degradation of Sulfur-Containing Aliphatic Glucosinolates in Broccoli Sprouts (*Brassica oleracea* var. *italica*) and Model Systems

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ABSTRACT: Processing reduces the glucosinolate (GSL) content of plant food, among other aspects due to thermally induced degradation. Since there is little information about the thermal stability of GSL and formation of corresponding breakdown products, the thermally induced degradation of sulfur-containing aliphatic GSL was studied in broccoli sprouts and with isolated GSL in dry medium at different temperatures as well as in aqueous medium at different pH values. Desulfo-GSL have been analyzed with HPLC-DAD, while breakdown products were estimated using GC-FID. Whereas in the broccoli sprouts structural differences of the GSL with regard to thermal stability exist, the various isolated sulfur-containing aliphatic GSL degraded nearly equally and were in general more stable. In broccoli sprouts, methylsulfanylalkyl GSL were more susceptible to degradation at high temperatures, whereas methylsulfinylalkyl GSL were revealed to be more affected in aqueous medium under alkaline conditions. Besides small amounts of isothiocyanates, the main thermally induced breakdown products of sulfur-containing aliphatic GSL were nitriles. Although they were most rapidly formed at comparatively high temperatures under dry heat conditions, their highest concentrations were found after cooking in acidic medium, conditions being typical for domestic processing.

KEYWORDS: *glucosinolates, thermally induced degradation, nitriles, isothiocyanates, structure−reactivity relationship, roasting, cooking, broccoli sprouts (Brassica oleracea var. italica)*

1. INTRODUCTION

Brassica crops such as cabbage, broccoli, mustard, or rape contain glucosinolates (GSL) (*β*-thioglucoside-*N*-hydroxysulfates), sulfur-containing secondary plant metabolites with diverse (plant) physiological properties.[1,2] With regard to their chemical structure, GSL consist of a *β*-D-glucopyranose moiety linked via a sulfur atom to a (Z)-*N*-hydroximinosulfate ester and a variable aglycone side chain. According to the variable side chain, structures can be classified in aliphatic, aromatic, and heterocyclic (e.g., indole) GSL with nearly 200 GSL being reported so far.[3−5] GSL that contain sulfur in the side chain are predominant within the aliphatic GSL. Sulfur-containing aliphatic GSL can be subdivided into methylsulfanylalkyl GSL(S-II), methylsulfinylalkyl GSL (S-IV), and methylsulfonylalkyl GSL (S-VI) according to the oxidative state of the sulfur atom.[4] After tissue disruption GSL are degraded enzymatically by myrosinase (*β*-thioglucosidase, EC 3.2.1.147), an enzyme occurring in all GSL-containing plants, to a variety of breakdown products (isothiocyanates, nitriles, thiocyanates, epithionitriles, and oxazolidine-2-thiones) depending on the reaction conditions. Due to the presence of isothiocyanates, *Brassica* consumption is thought to be beneficial, owing to their ability to induce phase II detoxification enzymes such as the NADPH quinone oxidoreductase and glutathione-*S*-transferases.[6−10]

Food technological processing of *Brassica* vegetables such as cooking, blanching, microwaving, and canning has been shown to affect GSL content and myrosinase activity considerably.[11,12] The loss of GSL can be due to enzymatic hydrolysis, leaching into the heating medium (e.g., cooking water), or thermally induced breakdown of the GSL.[13] In the past, several studies have been performed to estimate the loss of GSL in vegetables after applying different domestic processing techniques.[14,15] The strong decrease of GSL concentration in the *Brassica* product was supposed to result from leaching effects, as more or less good recoveries of the GSL have been found in the cooking water.[16] The remaining loss of GSL was probably due to enzymatic and to thermally induced degradation of the GSL.[17−19] Unfortunately, only few studies considered the latter effects separately. Especially, the thermally induced degradation of individual GSL was mostly not really considered. Oerlemans et al.[20] studied the thermally induced degradation of GSL in red cabbage. They found indole GSL being more thermolabile than aliphatic GSL. Different matrices may also have an impact on thermally induced degradation of GSL in *Brassica* vegetables as

Received: November 24, 2011
Revised: February 3, 2012
Accepted: February 9, 2012
Published: February 9, 2012

dx.doi.org/10.1021/jf204830p | *J. Agric. Food Chem.* 2012, 60, 2231−2241

it was demonstrated by Dekker et al.[21] Depending on the kind of vegetable processed, the degradation rate of the GSL was influenced but not the order in which the GSL were degraded. In a previous study it was shown that the chemical structure of the GSL and the pH value affect greatly the thermal breakdown of individual GSL.[22] Within the group of sulfur-containing aliphatic GSL, differences in the thermal stability have been revealed. Methylsulfanylalkyl GSL were more susceptible to a heat treatment than methylsulfinylalkyl GSL.[22] As dominant thermally induced breakdown products of the GSL, nitriles as well as isothiocyanates have been described.[23,24] Especially isothiocyanates deriving from sulfur-containing aliphatic GSL, e.g. sulforaphane, the hydrolysis product of 4-(methylsulfinyl)-butyl GSL (glucoraphanin; 4-MSOB), seem to be very potent anticancer agents.[8,25] This was also reported for structural analogues such as alyssin [5-(methylsulfinyl)pentyl isothiocyanate].[10] Therefore, it is of great interest to know how these GSL will be affected by a thermal treatment and which (breakdown) products are primarily released. With regard to this, it would be possible to evaluate the physiological properties of the processed vegetables.

The aim of the present study was to investigate the influence of temperature and pH on the thermally induced GSL degradation as well as on the formation of their corresponding breakdown products. Further, broccoli sprouts and a model compound mixture have been compared in order to evaluate the influence of the matrix on the thermal degradation.

2. MATERIALS AND METHODS

2.1. Chemicals. Benzonitrile (phenyl-CN) (99%), DEAE-Sephadex A-25, 4-pentenenitrile (3-butenyl-CN) (97%), and sodium hydroxide p.a. were purchased from Sigma-Aldrich (Steinheim, Germany); hydrogen peroxide (30%, Suprapur), iron(II) sulfate heptahydrate (≥99%), magnesium and palladium matrix modifier for graphite furnace AAS, methanol, potassium dihydrogen phosphate (≥99%), sodium acetate (≥99%), sodium carbonate (≥99%), sodium chloride (≥99%), disodium hydrogen phosphate dihydrate (≥99%), and sodium sulfate (≥99%) were purchased from Merck (Darmstadt, Germany); arylsulfatase, isolated from *Helix pomatia*, was purchased from Roche-Diagnostics GmbH (Mannheim, Germany); 4-(methylsulfinyl)butyl GSL potassium salt (glucoraphanin; 4-MSOB) and 4-(methylsulfanyl)butyl GSL potassium salt (glucoerucin; 4-MTB) were purchased from PhytoLab GmbH & Co. KG (Vestenbergsgreuth, Germany); acetonitrile and imidazole (≥99%) were purchased from Roth (Karlsruhe, Germany); iron standard solution 1 g/L for AAS was purchased from Sigma-Aldrich (Steinheim, Germany); nitric acid 65% (p.a.) was purchased from Bernd Kraft GmbH (Duisburg, Germany); methylene chloride (≥99.8%) was purchased from VWR (Darmstadt, Germany). 4-Hydroxybenzyl GSL and isolated broccoli GSL (GI) were provided by the Leibniz-Institute of Vegetable and Ornamental Crops Grossbeeren/Erfurt e.V. (Grossbeeren, Germany). 4-Hydroxybenzyl GSL (sinalbin) was extracted and purified from white mustards seeds (*Sinapis alba*), whereas GI was isolated from seeds of broccoli (*Brassica oleracea* var. *italica*), both according to a modified method of Thies.[26] The GI contained 51% 4-MSOB, 25% 3-(methylsulfinyl)propyl GSL (glucoiberin; 3-MSOP), 13% 4-MTB, 4% 3-(methylsulfanyl)propyl GSL (glucoiberverin; 3-MTP), and traces of other GSL as potassium salts. Purity of the GSL mixture and of 4-hydroxybenzyl GSL was ≥99%, according to HPLC-DAD. All solvents were of HPLC grade. Water was of Milli-Q quality.

2.2. Plant Material. Broccoli (*Brassica oleracea* var. *italica*) sprouts cv. Calabrese were provided by the Leibniz-Institute of Vegetable and Ornamental Crops Grossbeeren/Erfurt e.V. (Grossbeeren, Germany). Seeds were sown on water soaked fleece in aluminum trays filled with perlite and water. They were atomized daily until germination by using a water sprayer. Water was given as needed for optimal sprout growth. Trays were kept in a greenhouse at about 24 °C during the day and 20 °C during the night with a relative humidity of about 75%. Supplemental irradiation of 150 μmol m^{-2} s^{-1} was provided to give a 16 h photoperiod. Sprouts were harvested being 12 days old, lyophilized, and ground to a fine powder.

2.3. Roasting. For the dry heat treatments, either 200 mg of broccoli sprout powder or 2−3 mg of extracted broccoli GSL (GI) was weighed into a glass ampule and sealed immediately. The heat treatment was carried out at 100 or 130 °C in a behrotest ET2 thermoblock (Behr Labortechnik, Germany). Sampling of GI was done after 0, 10, and 30 min. Broccoli sprouts were treated 0, 10, 30, 45, and 60 min. All treatments were done in triplicate. The samples were cooled on ice and extracted for analysis, or GI was dissolved in 2 mL of water and applied directly on a DEAE-Sephadex A-25 ion exchanger column for GSL analysis.

2.4. Cooking. *2.4.1. Broccoli Sprouts.* 150 mg of broccoli sprouts was weighed into a 20 mL crimp vial and preheated at 100 °C for 1 min on a heating element. Exactly 4 mL of hot water (for pH 5.3) or of a hot Na_2CO_3/$NaHCO_3$ buffer solution (pH 9.35) (for pH 8.0 treatments) was added to the broccoli sprouts. Vials were immediately sealed and finally treated at 100 °C in the thermoblock. Sampling was done after 0, 10, 30, 45, and 60 min. To test for an influence of Fe^{2+} ions, the water was spiked with 0.18 μmol or 0.018 μmol of a solution in water of iron(II) sulfate, simulating the addition of the 1.3-fold soluble and overall iron of the broccoli sprouts. These samples were cooked for 45 min. Immediately, samples were cooled on ice and, for GSL analysis, frozen at −30 °C prior to lyophilization. Extraction and GC-FID analysis of the breakdown products was done instantly (as described in section 2.6). For a comparison with untreated samples, 4 mL of the buffer solution was freeze-dried and afterward mixed with 150 mg of broccoli sprouts. Each sample was treated in triplicate.

2.4.2. GSL Extract (GI). 2−3 mg of GI was weighed into crimp vials, and 1.5 mL of a hot KH_2PO_4/Na_2HPO_4 buffer solution (pH 5.3 and pH 8.0) was added. Treatment was conducted as described for the cooking of the broccoli sprouts. Sampling was done after 10 and 30 min in 4-fold. Treated GI was desulfated afterward for GSL analysis or extracted for GC-FID analysis of breakdown products as described later in the text (sections 2.5 and 2.6).

2.5. Extraction of GSL and HPLC-DAD Analysis of Desulfo-GSL. A modified method of Mewis et al.[27] was used for the HPLC analysis of the GSL. Briefly, 30 mg of broccoli sprouts was heated to 75 °C and incubated for 1 min, extracted for 10 min with 1 mL of methanol/water mixture (v/v 7:3, t = 75 °C), and centrifuged at 18620g for 5 min. 100 μL of a 1 mM stock solution of the internal standard 4-hydroxybenzyl GSL (sinalbin) was added prior to the first extraction. The residue was extracted two further times with 0.75 and 0.5 mL of the hot methanol/water mixture. The supernatants were combined and applied on a 150 μL DEAE-Sephadex A-25 ion exchanger column, prepared and washed according to the method of Mewis et al.[27] Next, 100 μL of a purified arylsulfatase solution was applied to the column and left for 12 h before the desulfo-GSL were eluted with 1.5 mL of water.

For the analysis of cooked broccoli sprouts, samples at pH 8 and for the cooked GI a modified method of Krumbein et al.[28] was used. Freeze-dried material (30 mg), consisting of a mixture of freeze-dried buffer and broccoli sprouts, was extracted three times as described above. The pooled extract or the cooked GI samples were applied on a 125 μL DEAE-Sephadex A-25 ion exchanger column (acetic acid activated), rinsed with 2 mL of water, desulfated, and eluted as described above.

Desulfated extracts were separated and analyzed by HPLC-DAD as described previously.[22] Separation was carried out on a ProntoSIL Spheribond ODS2 column (3 μm, 125 mm × 4 mm) with a gradient consisting of water (A) and acetonitrile (B) and a flow rate of 0.7 mL min^{-1}. GSL were detected at a wavelength of 229 nm. Their content was calculated using 4-hydroxybenzyl GSL as internal standard and the response factor of each compound relative to 4-hydroxybenzyl GSL.

2.6. Extraction of GSL Degradation Products and Analysis Using GC-FID. For the determination of the thermally induced breakdown products of the GSL, the method reported by Lambrix et al.[29] was adapted. To 200 mg of the roasted broccoli sprouts (respectively the 150 mg of the cooked sprouts or the treated GI) 2.5 mL of methylene chloride was added. Samples were quantitatively transferred to a 10 mL glass tube. 100 μL of a 1 mM solution of phenyl-CN in methylene chloride as internal standard was added, and the tubes were sealed. After shaking for 20 s and centrifugation at 2330*g* for 10 min, the methylene chloride layer was filtered through a small column of anhydrous sodium sulfate to remove residual water. The remaining aqueous layer or residue was re-extracted with 1 mL of methylene chloride. The dried extracts were combined, concentrated under a nitrogen gas flow to 300 μL, and transferred into a vial.

Samples were analyzed by gas chromatography flame ionization detection (GC-FID) using a Hewlett-Packard 5890 A GC equipped with a BP 5 column (30 m × 0.25 mm × 0.25 μm film). After splitless injection of 1 μL of the sample at 190 °C, analytes were separated, using He as carrier gas (1.8 mL min^{-1}). A temperature gradient starting at 35 °C for 3 min and raising up to 230 °C with 9 °C min^{-1} was used for separation. Analyte content was calculated using phenyl-CN as internal standard and the response factor (RF) of each compound relative to phenyl-CN. The RF were calculated according to the effective carbon number (ECN) concept.[30]

2.7. Thermal Stability of Nitriles. Phenyl-CN and 3-butenyl-CN were cooked at pH 5 or pH 8 or treated at 130 °C to test for the thermal stability of the nitriles. Cooking was done as described above for the isolated broccoli GSL (section 2.4). 10 μmol of phenyl-CN or 1 μmol of 3-butenyl-CN was cooked, and sampling was done in triplicate after 10 and 60 min. For the treatment at 130 °C, 50 μL of each substance was filled in vials and diluted afterward with methanol. Dilutions of the phenyl-CN samples were analyzed with the HPLC-DAD as described above (section 2.5) with a gradient, consisting of 1−35% acetonitrile in water from 2 to 10 min, increasing up to 70% acetonitrile in water until 34 min. Determination of phenyl-CN was conducted at a flow rate of 0.5 mL min^{-1}, a column temperature of 30 °C, and a wavelength of 240 nm. 3-Butenyl-CN was analyzed by GC-FID as described above (section 2.6). Dilutions of the roasted samples were injected without further cleanup. Cooked samples were extracted with methylene chloride using phenyl-CN as internal standard as was described above (section 2.6).

2.8. Analysis of Iron by Graphite Furnace AAS. Iron was analyzed to estimate whether there could be an influence of iron toward a change in the thermally induced breakdown product profile. Total iron was determined by graphite furnace AAS analysis. Therefore, 20 mg of broccoli sprouts was digested with 500 μL of a mixture of hydrogen peroxide (30%) and concentrated nitric acid (v/v 1:1) in a shaking heater at 95 °C to dryness. The residue was dissolved in 1 mL of water and diluted with 0.14 N nitric acid. For determining soluble iron in the samples, 35 mg of broccoli sprouts was extracted with 1 mL of 0.0014 N nitric acid (pH 2.7) for 10 min by ultrasonic treatment. After centrifugation at 18620*g* for 5 min, the residue was re-extracted with 1 mL of the 0.0014 N nitric acid. The combined extract was dried at 95 °C in a shaking heater. The residue was digested as described above and used for the AAS analysis of the soluble iron. GI did not need sample preparation. Residues or 3 mg of GI was dissolved in 1 mL of water and diluted with 0.14 N nitric acid. Each sample was prepared in triplicate. Graphite furnace AAS was conducted with a PerkinElmer AA800 atomic absorption spectrophotometer, PerkinElmer AS800 autosampler, and the software WinLab32 for AA, version 6.4.0.0191. Calibration was performed with dilutions of an iron standard solution of 1 g L^{-1}, and magnesium and palladium matrix modifier for graphite furnace AAS were used for each measurement.

2.9. Statistical Analysis. For the analysis of variance, Tukey's honest significant difference (HSD) test was used to calculate differences at a significance level of $p \leq 0.05$ (one-way ANOVA for single influences, two-way ANOVA to test for interactions). It was performed to test the nitrile results for significance of the influence of temperature or pH on their formation as well as the GSL results for significance of the influence of structure of the GSL, treatment, matrix (see section 3.1.1), and structure × treatment on the thermally induced degradation by using Origin Pro 8.0 (OriginLab Corporation, Northampton, MA, USA). Data in figures were reported as mean ± SD (broccoli sprouts, $n = 3$; GI, dry treatment $n = 3$, cooking $n = 4$).

3. RESULTS

3.1. Thermally Induced Degradation of GSL in Broccoli Sprouts and Formation of Their Corresponding Breakdown Products. In a previous study differences between the thermal susceptibility of especially the five sulfur-containing aliphatic GSL found in broccoli sprouts have been reported.[22] In the study presented here, the focus was on the development of breakdown products formed resulting from a thermally induced degradation of the GSL depending on different thermal treatments. Therefore, breakdown of the sulfur-containing aliphatic GSL (Figure 1) [3-(methylsulfanyl)propyl

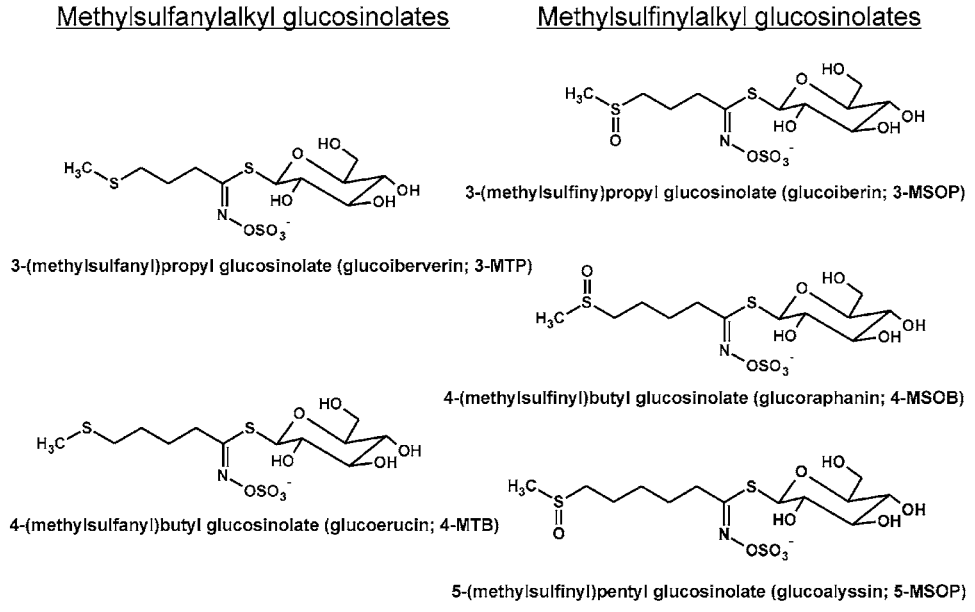

Figure 1. Chemical structures of sulfur-containing aliphatic glucosinolates in broccoli sprouts.

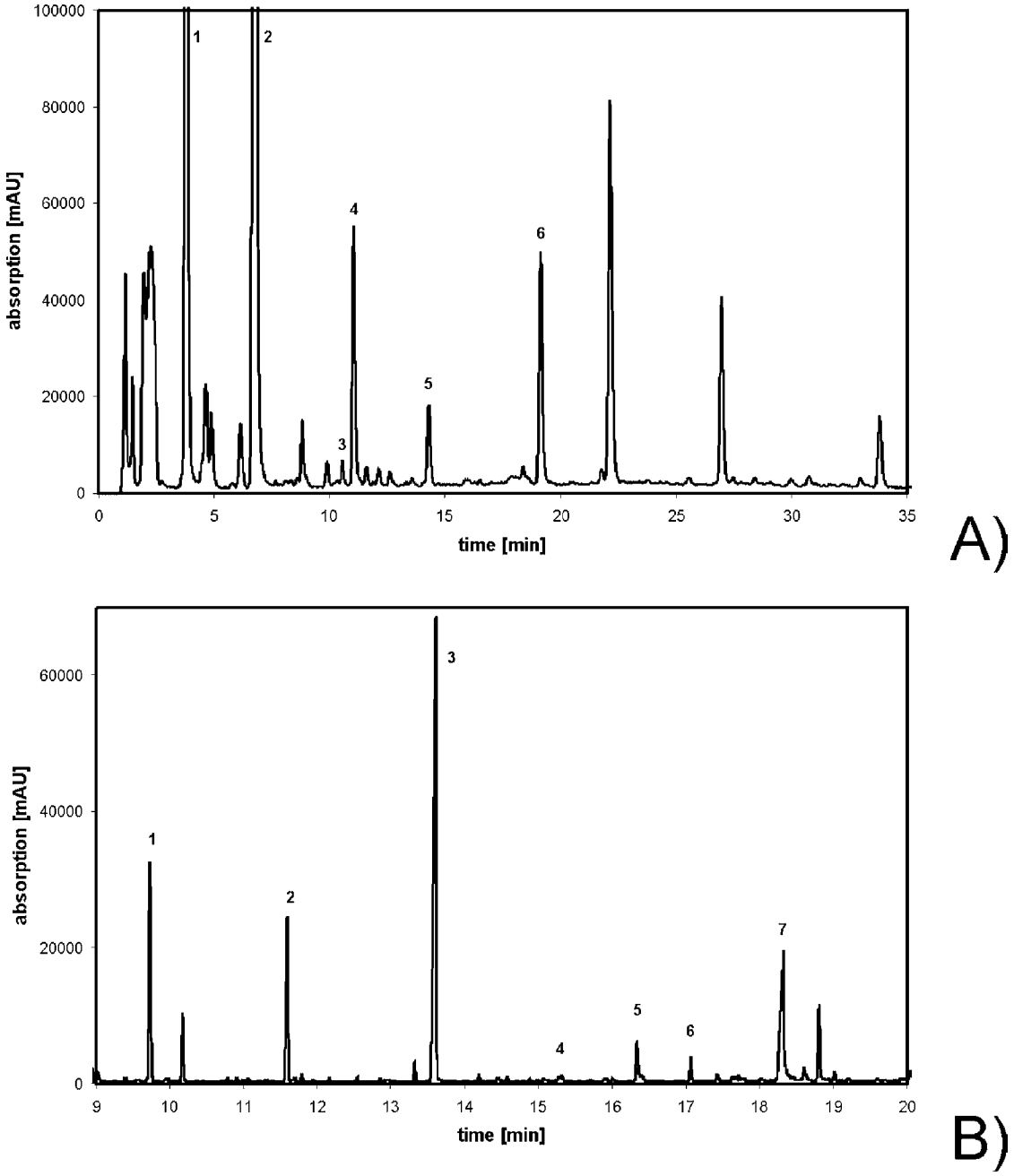

Figure 2. (A) HPLC chromatogram of desulfo glucosinolates (GSL) (229 nm) of a cooked broccoli sprout sample (60 min, pH 5.3): (1) 3-(methylsulfinyl)propyl GSL, (2) 4-(methylsulfinyl)butyl GSL, (3) 5-(methylsulfinyl)pentyl GSL, (4) 4-hydroxybenzyl GSL (internal standard), (5) 3-(methylsulfanyl)propyl GSL, (6) 4-(methylsulfanyl)butyl GSL. (B) GC-FID chromatogram of breakdown products of a cooked broccoli sprout sample (60 min, pH 5.3): (1) benzonitrile (internal standard), (2) 4-methylsulfanylbutylnitrile, (3) 5-methylsulfanylpentylnitrile, (4) 3-methylsulfanylbutyl isothiocyanate, (5) 4-methylsulfinylbutylnitrile, (6) 4-methylsulfanylbutyl isothiocyanate, (7) 5-methylsulfinylpentylnitrile.

GSL (glucoiberverin; 3-MTP), 3-(methylsulfinyl)propyl GSL (glucoiberin; 3-MSOP), 4-(methylsulfanyl)butyl GSL (glucoerucin; 4-MTB), 4-(methylsulfinyl)butyl GSL (glucoraphanin; 4-MSOB), 5-(methylsulfinyl)pentyl GSL (glucoalyssin; 5-MSOP)] was studied by applying dry heat at different temperatures, as some of the *Brassica* species are common to be roasted (e.g., rapeseed), and heating under aqueous conditions at different pH values. The formation of the corresponding breakdown products was analyzed by GC-FID. Broccoli sprouts contained 2.2 μmol of 3-MTP g^{-1} DW (dry weight), 12.8 μmol of 3-MSOP g^{-1} DW, 6.4 μmol of 4-MTB g^{-1} DW, 31.6 μmol of 4-MSOB g^{-1} DW, and 0.3 μmol of 5-MSOP g^{-1} DW as base levels prior to the treatments. Figure 2 shows a typical HPLC chromatogram of the separation of desulfo-GSL and a typical GC-FID chromatogram of breakdown products from a cooked broccoli sprout sample. Sulfur-containing aliphatic GSL were degraded and produced corresponding nitriles and isothiocyanates, whereby the nitriles were always the dominating breakdown products. Figures 3 and 4 show the degradation of selected sulfur-containing aliphatic GSL in broccoli sprouts and the formation of nitriles in dependence of the different treatments. Isothiocyanates were formed only in comparatively small amounts, and only those of methylsulfanylalkyl GSL were found at longer treatment times and not at 130 °C. At 100 °C

dx.doi.org/10.1021/jf204830p | J. Agric. Food Chem. 2012, 60, 2231–2241

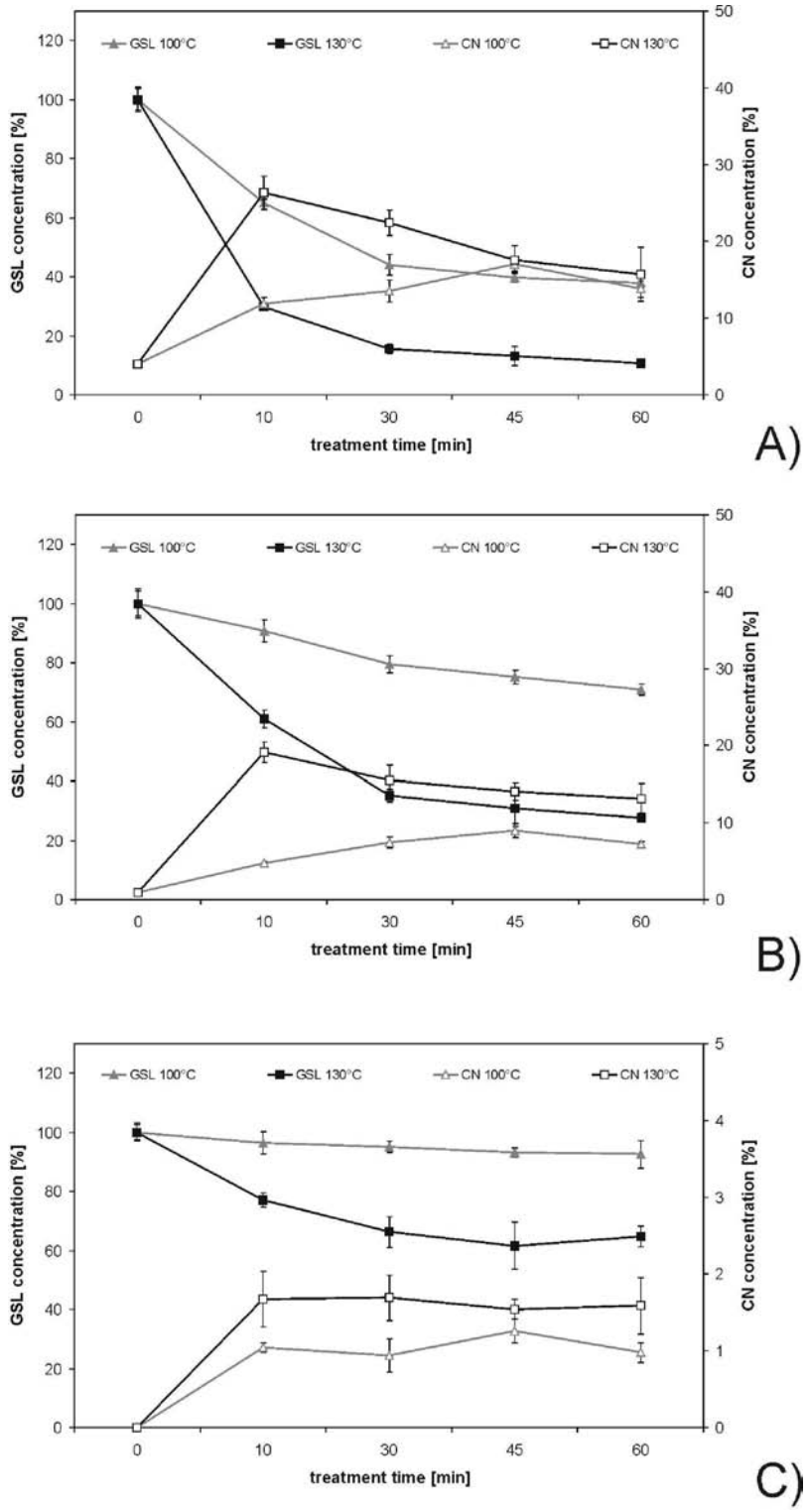

Figure 3. Thermally induced degradation of sulfur-containing aliphatic glucosinolates (GSL) from broccoli sprouts {(A) 3-MTP [3-(methylsulfanyl)propyl GSL], (B) 4-MTB [4-(methylsulfanyl)butyl GSL], (C) 4-MSOB [4-(methylsulfinyl)butyl GSL]} and formation of the corresponding nitriles under roasting conditions (100 and 130 °C). GSL breakdown was analyzed by HPLC-DAD and formation of breakdown products by GC-FID. Results were expressed as remaining GSL in % of the initial content, nitriles (CN) in % of the initial GSL content, respectively.

they only accounted for 2% of the overall analyzed breakdown products resulting from each of the methylsulfanylalkyl GSL (data not shown). In contrast to the dry conditions, in aqueous medium 3-methylsulfanylpropyl isothiocyanate accounted for up to 19%, 4-methylsulfanylbutyl isothiocyanate for up to 13% of the detected breakdown products of the corresponding GSL and the concentrations decreased after 45 min of treatment time (data not shown).

3.1.1. Influence of the Temperature on Thermal Breakdown of GSL. To investigate the influence of temperature on the thermally induced degradation of the GSL in broccoli sprouts, they have been treated in dry heat at two different

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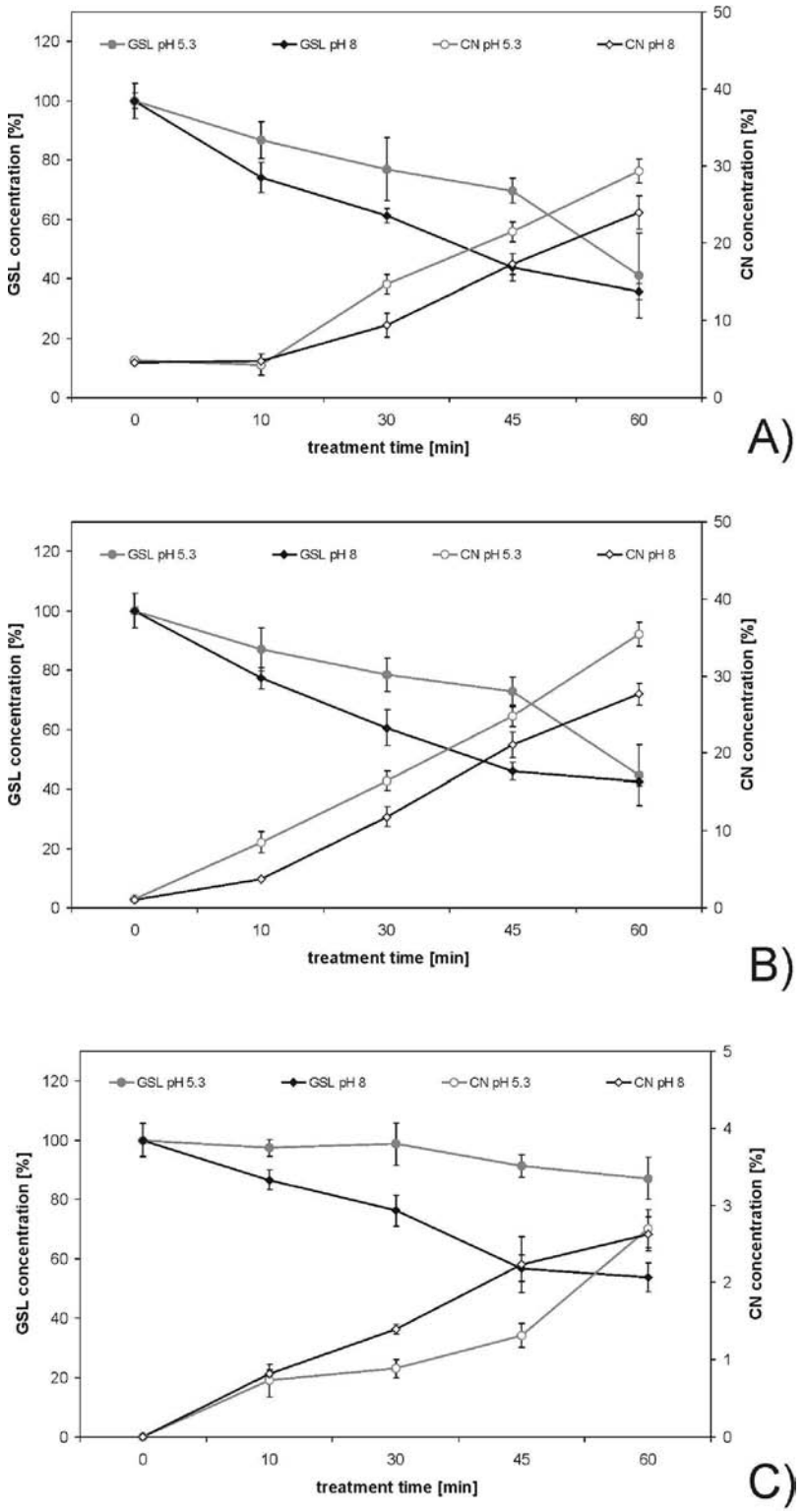

Figure 4. Thermally induced degradation of sulfur-containing aliphatic GSL from broccoli sprouts {(A) 3-MTP [3-(methylsulfanyl)propyl GSL], (B) 4-MTB [4-(methylsulfanyl)butyl GSL], (C) 4-MSOB [4-(methylsulfinyl)butyl GSL]} and formation of the corresponding nitriles in aqueous medium (100 °C, pH 5.3 and pH 8). GSL breakdown was analyzed by HPLC-DAD and formation of breakdown products by GC-FID. Results were expressed as remaining GSL in % of the initial content, nitriles (CN) in % of the initial GSL content, respectively.

temperatures (100 and 130 °C). In Figure 3A–C the degradation of 3-MTP (A), 4-MTB (B) and 4-MSOB (C), as well as the formation of the corresponding nitriles 4-methylsulfanylbutylnitrile (3-MTP-CN), 5-methylsulfanylpentylnitrile (4-MTB-CN), and 5-methylsulfinylpentylnitrile (4-MSOB-CN), is illustrated. The thermal degradation of 3-MSOP and 5-MSOP and the formation of the 4-methylsulfinylbutylnitrile (3-MSOP-CN) were also monitored and showed a similar trend comparable to 4-MSOB-CN. Breakdown products of 5-MSOP have not been found as the concentration of this GSL is very low in broccoli sprouts.

dx.doi.org/10.1021/jf204830p | J. Agric. Food Chem. 2012, 60, 2231–2241

At 100 °C the methylsulfanylalkyl GSL were significantly degraded with 3-MTP being the most susceptible one (62% degradation after 60 min) (Figure 3A). In contrast, the methylsulfinylalkyl GSL were comparatively stable with only 7% degradation of 4-MSOB after 60 min (Figure 3C). Resulting from the dry heat treatments, the corresponding nitriles were found as breakdown products. In Figures 3 and 4 the relative concentration of nitriles is presented in % referring to the initial concentration of the corresponding GSL. At 100 °C, the highest nitrile concentrations were found after 45 min, afterward declining slightly (Figure 3A,B). However, the recovery rates (percentage of the recovered breakdown product of the degraded GSL) of the nitriles reached from 13% (4-MSOB-CN, 60 min) to 51% (4-MTB-CN, 10 min), were highest after 10 min, and decreased after this treatment time.

At a temperature of 130 °C, the breakdown of the GSL was much more pronounced. 3-MTP was degraded to 89% and 4-MTB to 72% after one hour treatment. The methylsulfinylalkyl GSL were more stable: 35% of 4-MSOB and 3-MSOP were degraded after 60 min at 130 °C. In comparison to 100 °C, the concentrations of nitriles for the studied GSL treated at 130 °C were higher and reached their maximum levels after 10 min of treatment. Up to 26% of the initial concentration of 3-MTP was found as its corresponding nitrile after 10 min. In the further course of the treatment, the concentrations of nitriles decreased or were more or less stable as in the case of the 4-MSOB-CN. The recovery rates were highest after 10 min, similarly to the results of the 100 °C treatments. 4-MTB-CN had the highest recovery rate with 49% after 10 min. 4-MSOB-CN had a very low recovery at 130 °C (5−7%) compared to 100 °C (13−29%), although the GSL showed significant thermally induced degradation. In contrast to the 100 °C treatments, degradation of the nitriles was more pronounced after 10 min, whereas after 60 min the concentrations of 3-MTP-CN were the same for both temperatures.

3.1.2. Influence of the pH Value on Thermally Induced Degradation of GSL under Aqueous Conditions. Broccoli sprouts were cooked in water without any change of the initial pH value of 5.3 during the treatment. Due to the cooking procedure, enzyme catalyzed breakdown of GSL was negligible in this experiment (enzyme inactivation). Further, sprouts were treated in a carbonate buffer (pH 9.35), to study exemplarily the influence of a shift in the pH on the thermal degradation of GSL. In contrast to the original pH value of the broccoli sprouts (pH 5.3), here, the pH shifted to a value of 8.0, remaining stable during the further course of the cooking procedure. In Figure 4A−C, the thermally induced degradation of GSL resulting from cooking is shown for 3-MTP (A), 4-MTB (B) and 4-MSOB (C). Additionally, the formation of the corresponding nitriles 3-MTP-CN, 4-MTB-CN, and 4-MSOB-CN is presented. The two methylsulfanylalkyl GSL showed similar stability (Figure 4A,B). Both are more thermolabile at pH 8.0, but this effect disappears at 60 min and the degradation kinetics were equal at both pH values. Although they were degraded preferably in the basic medium, the formation of nitriles was higher at pH 5.3. In this weak acidic medium, the recovery rates of 4-MTB-CN were very high with 63% (60 min) to 91% (45 min). The recovery of 3-MTP-CN was a bit lower, ranging from 32% (10 min) to 71% (45 min). However, at basic conditions the formation of the methylsulfanylalkylnitriles was less and the recovery rates shifted from only 16% to 47%.

In contrast to the methylsulfanylalkyl GSL, the methylsulfinylalkyl GSL were comparatively stable at pH 5.3. However, the methylsulfinylalkyl GSL were by far more affected by a treatment under basic conditions (see also Figure 5A,C). The course of the degradation at pH 5.3 and pH 8 did not adapt, and after 60 min 53% of the 4-MSOB had been degraded (Figure 4C). In contrast to the methylsulfanylalkyl GSL, where more nitriles were formed in the acidic medium, up to 45 min more methylsulfinylnitrile formation occurred in the basic medium. Nevertheless, at a later time, at 60 min, no differences in the nitrile concentrations were observed and at both pH values the same concentrations of 4-MSOB-CN were found. On the other hand, the recovery of 4-MSOB-CN was, like that of the methylsulfanylalkylnitriles, higher at pH 5.3, ranging from 15% (45 min) to 29% (10 min), compared to pH 8, where it was 5−6%.

3.2. Effect of Matrix and of Treatment on Thermal Degradation of GSL. In Figure 5A the remaining GSL concentrations in the broccoli sprouts after 30 min of the different thermal treatments are shown. As reported in a previous work, the methylsulfinylalkyl GSL are generally more stable in comparison to their corresponding methylsulfanylalkyl GSL. Additionally, during the dry heat treatments, 4-MTB was more stable compared to 3-MTP as reported before.[22] On the other hand, after the thermal treatments in aqueous media, the contribution of the side chain to the thermal stability of the sulfur-containing aliphatic GSL in the broccoli sprouts could not be verified. There was no difference between 3-MTP and 4-MTB, only between methylsulfanylalkyl and methylsulfinylalkyl GSL, which are comparatively more stable (Figure 5A,C).

To evaluate the influence of the matrix toward the thermally induced degradation, a mixture of extracted and purified GSL was treated similarly to the broccoli sprouts. In Figure 5B the remaining GSL concentration in this mixture after 30 min of treatment is shown. At 100 °C, only the methylsulfanylalkyl GSL were slightly degraded with only 15% of degradation of 3-MTP. At 130 °C, methylsulfanylalkyl as well as methylsulfinylalkyl GSL degraded in the same manner to about 50−55%. In the slightly acidic aqueous medium, only 3-MTP was degraded to a little extent. In basic medium there has not been observed any degradation of the GSL. To prove an influence of the plant matrix on the thermal stability of the GSL, one-way ANOVA was performed comparing the degradation of the GSL in the broccoli sprouts with the isolated broccoli GSL after 30 min of each treatment (Figure 5A,B). In general, isolated methylsulfanylalkyl GSL were always significantly more stable as compared to the ones in broccoli sprouts, with the exception of 3-MTP after cooking at pH 5.3, where no difference could be observed between the different samples. The methylsulfinylalkyl GSL were equally stable at 100 °C and pH 5.3 (no detected degradation), but significantly less stable at 130 °C when compared to the GSL in broccoli sprouts. Nitriles were, similarly to the results of the broccoli sprout treatments, the main thermal breakdown products of the isolated broccoli GSL. Breakdown products of the methylsulfanylalkyl GSL were found predominantly, but in comparison with the broccoli sprouts, higher percentages of isothiocyanates as breakdown products were detected at 100 and 130 °C. In contrast to the broccoli sprouts, the recovery rates of the nitriles were lower at 100 °C versus 130 °C.

In Figure 5C the influence of the different heat treatments on the stability of each GSL in broccoli sprouts after 45 min of treatment is shown. Methylsulfanylalkyl GSL were significantly less stable at 130 °C and most stable at a pH value of 5.3 during cooking. 3-MTP showed the same stability at

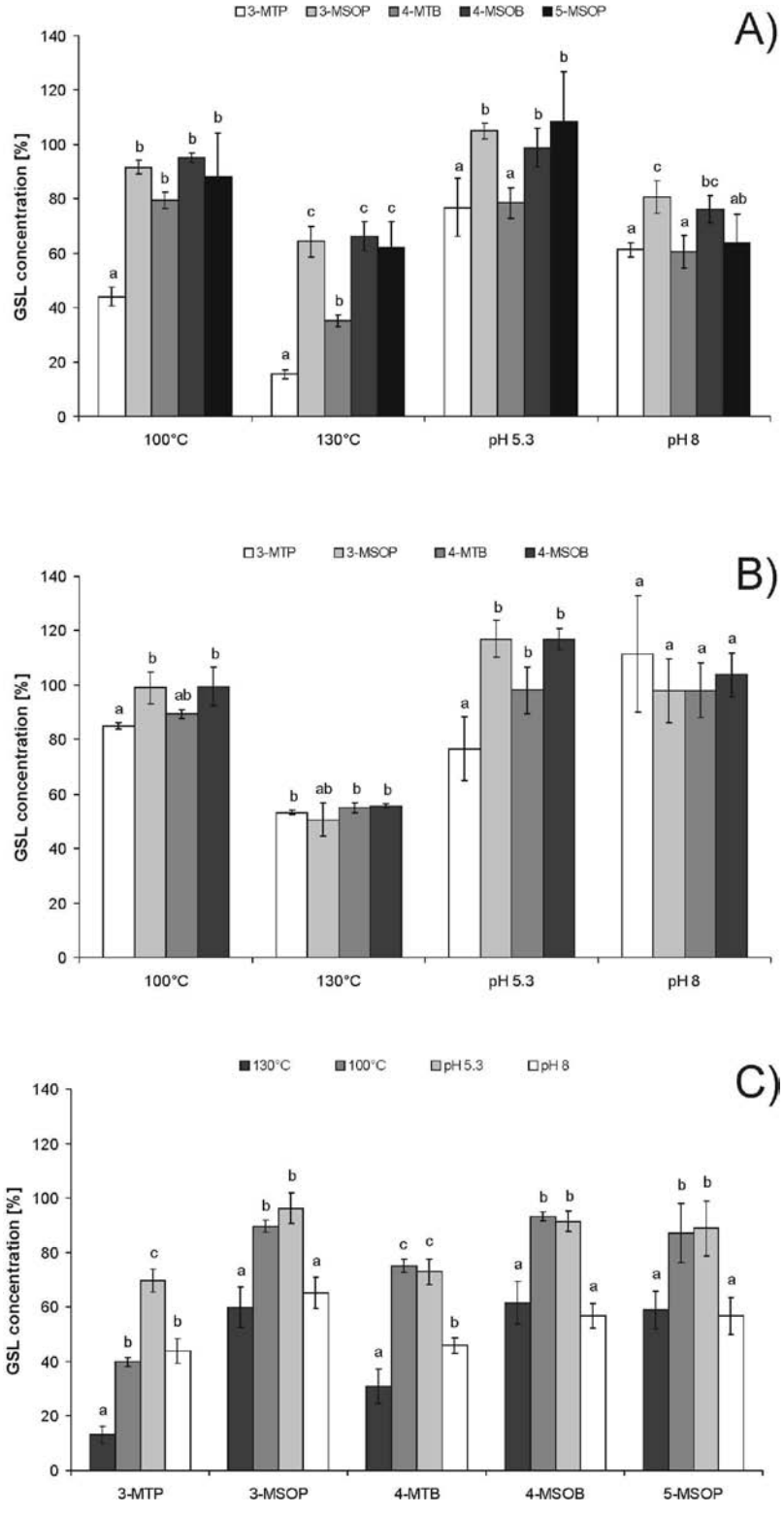

Figure 5. Glucosinolate (GSL) concentrations of thermally induced degradation of sulfur-containing aliphatic GSL in dependence of the plant matrix and treatments: (A) broccoli sprouts after 30 min of treatment; (B) isolated GSL mixture after 30 min of treatment; (C) broccoli sprouts after 45 min of treatment. GSL breakdown was analyzed by HPLC-DAD. 3-MTP, 3-(methylsulfanyl)propyl GSL; 3-MSOP, 3-(methylsulfinyl)propyl GSL; 4-MTB, 4-(methylsulfanyl)butyl GSL; 4-MSOB, 4-(methylsulfinyl)butyl GSL; 5-MSOP, 5-(methylsulfinyl)pentyl GSL. Results were expressed as remaining GSL in % of the initial content. Different small letters in each chart indicate significant differences between the structures (panels A and B), tested individually at each treatment, or between each treatment tested individually for each substance (panel C) ($p \leq 0.05$ by Tukey's HSD test).

100 °C and a pH value of 8.0. On the other hand the methylsulfinylalkyl GSL were always less stable at pH 8.0 or 130 °C and most stable when cooked at pH 5.3 or treated at 100 °C for 45 min as well as 60 min. The two-way ANOVA analysis revealed significant interaction between treatment and the GSL structure.

3.3. Thermal Stability of Nitriles. As the above-mentioned results lead to the hypothesis that the nitriles seemed to be not stable under the treatment conditions applied, their stability was evaluated in a further model approach using benzonitrile (phenyl-CN) and 4-pentenenitrile (3-butenyl-CN), being the hydrolysis product of the widespread GSL gluconapin. Phenyl-CN showed a slight decrease due to the thermal influence, but no breakdown products could be detected. It was most stable at 130 °C with 93.7% ± 4.5% being left over after 60 min. At pH 5.3 still 91.5% ± 1% was detectable after the whole treatment time, and at pH 8.0 89% ± 0.1% was still present. 3-Butenyl-CN was more labile compared to phenyl-CN, but was significantly more degraded at 130 °C. It was most stable at pH 5.3 with 90.8% ± 8.7% being detectable after 60 min. At pH 8.0 still 88.1% ± 7.4% was left, and at 130 °C 79.3% ± 4.6% was still detectable.

3.4. Influence of Iron on the Thermal Breakdown of GSL. As Fe(II) ions are able to degrade GSL nonenzymatically[34] and thermal breakdown of GSL differed between the broccoli sprouts and the isolated broccoli GSL (GI), the influence of iron on the thermally induced degradation of the GSL was evaluated. Soluble and total iron was determined by graphite furnace AAS in the broccoli sprouts and also in the GI. No iron could be detected in the GI. Broccoli sprouts contained 0.92 ± 0.37 μmol of Fe g^{-1} DW of total iron. 0.09 ± 0.01 μmol of Fe g^{-1} DW of this iron was soluble. Addition of the 1.3-fold of the soluble or total iron to broccoli sprouts significantly reduced the thermal stability of the GSL after 45 min of cooking and led to an enhanced nitrile production. The stability of the sulfur containing aliphatic GSL was reduced with higher iron concentration for approximately 4−7%. Nitrile concentrations were doubled with addition of the soluble iron and tripled when adding the total iron concentration. No isothiocyanates could be detected in these samples.

4. DISCUSSION

The influence of different thermal treatments on the degradation of sulfur-containing aliphatic GSL and the formation of their corresponding breakdown products was studied either with broccoli sprouts or with a GSL mixture isolated from broccoli. GSL are according to their structure susceptible to thermally induced degradation. Indole GSL are in contrast to the aliphatic GSL considered to possess the most heat sensitive structures.[20,22,32] As discussed recently, there is an influence of the oxidation state of the sulfur in the side chain of the sulfur-containing GSL with regard to their thermal stability: Methylsulfinylalkyl GSL are more stable in comparison to their corresponding methylsulfanylalkyl GSL.[22] From the five sulfur-containing aliphatic GSL tested, 3-MTP was always the most thermolabile one. Michajlovskij et al. also revealed this GSL being less stable toward cooking.[33] Volden et al. found significant losses of 4-MTB after cooking cauliflower.[12] However, some studies suggested 3-MSOP to be more thermolabile than others, even more than 1-indolylmethyl GSL.[17] But in the study of Oerlemans et al. 3-MSOP and 4-MSOB were more stable during cooking at 100 and 120 °C compared to the indole GSL.[20] Additionally, Hanschen et al. observed after dry heat treatments that within the methylsulfanylalkyl GSL a longer side chain stabilizes the GSL against thermal degradation.[22]

In the present study, broccoli sprouts were treated under dry conditions at 100 and 130 °C or were cooked at pH values of 5.3 or 8.0 and GSL degradation as well as breakdown product formation was analyzed in order to identify main influencing factors. In general, the influence of the oxidation state toward thermally induced degradation of GSL was observed as reported before.[22] However, during the dry heat experiment (roasting), the destabilizing influence of a longer side chain within the group of methylsulfinylalkyl GSL, that was reported before for roasting at 130 °C, could not be verified in the present study. One reason could be that in this approach all treatments were conducted in closed glasses and the occurring pressure might influence the thermally induced degradation.

Further, in the aqueous heat experiments (cooking), differences in the thermal stability within the group of methylsulfanylalkyl GSL, resulting from the side-chain length, were not observable and seem not to be valid for treatments in aqueous media (Figure 4A,B). Both methylsulfanylalkyl GSL (3-MTP and 4-MTB) were equally stable whereas the methylsulfinylalkyl GSL were still more stable than the methylsulfanylalkyl GSL. The influence of the oxidation state was still existent. As was shown by the two-way-ANOVA evaluation, the sulfur-containing aliphatic GSL are affected differently by the various treatments. This might be due to different mechanisms the GSL may undergo resulting from the thermally induced degradation.

The accelerated degradation of the GSL in broccoli sprouts in comparison to the isolated GSL, which were comparatively more stable during the treatments, might be attributed to the presence of iron, occurring in small amounts in the broccoli sprouts as shown by graphite furnace AAS. It is known that Fe(II) ions, in contrast to Fe(III) ions, can influence the degradation of GSL nonenzymatically to form nitriles.[24,34] Higher temperature accelerates the Fe(II)-induced degradation, and also at lower Fe^{2+} concentrations (0.1 mol of Fe^{2+} (mol of GSL)$^{-1}$) degradation occurs.[35] In this study it was shown that addition of even little amounts of iron significantly reduced the thermal stability of the GSL in broccoli sprouts during cooking at pH 5.3 and increased the formation of the corresponding nitriles. But, as this iron-induced degradation is thought to preferentially take place in acidic medium, degradation in basic medium cannot be explained, as iron is weakly soluble under basic conditions. However, other substances in the plant matrix might influence the thermal degradation of GSL as well.

The detection of isothiocyanates and nitriles as thermally induced breakdown products of the GSL is in accordance with earlier studies. MacLeod and MacLeod suggested nitriles being the most dominant thermally induced breakdown products.[36] Later, they observed the formation of both nitriles and isothiocyanates, after injecting GSL into a gas chromatograph: Only at higher column temperature isothiocyanate formation occurred.[23] In the study presented here, little amounts of isothiocyanates, especially after cooking the broccoli sprouts and after the dry treatments at 100 °C, have been detected. However, when heating the isolated GSL, a higher isothiocyanate/nitrile ratio has been detected at 100 °C. Although more isothiocyanates were produced at 130 °C, the ratio of the isothiocyanates on the breakdown products was much lower due to the comparatively higher nitrile concentration. So far, it was thought that isothiocyanate formation is favored by greater heat,[23] but the present study revealed that relative isothiocyanate contents decreased with increasing thermal impact.

During the roasting of the broccoli sprouts decreased nitrile concentrations and lower recovery rates for the nitriles have been observed with longer treatment time and higher temperature. As an evaporation of the breakdown products was

excluded in this approach, this indicates that the nitriles seem to be not stable under such conditions and are further degraded or they are able to react with other constituents of the plant matrix. Especially the strong decrease of the 3-MTP-CN after 10 min at 130 °C indicates further reactions. In contrast to the results from the dry heat treatments, the formation of nitriles is favored in an aqueous medium. Regardless of the pH, the concentrations of nitriles did not reach a satiety level after 60 min (Figure 4) and probably with longer treatment times higher nitrile concentrations might have occurred. With regard to all the treatments, the highest amounts of nitriles were found in the aqueous medium when treated at least for 45 min (Figures 3 and 4). Probably, nitriles are more stable under such conditions. On the other hand, at the basic pH values, the recovery rates were much lower compared to the acidic medium. All in all, methylsulfinylalkylnitriles showed lower recovery rates compared to the methylsulfanylalkyl nitriles. Maybe their thermal instability/reactivity is greater compared to the methylsulfanylalkylnitriles.

After the dry heat treatments of the isolated GSL mixture, the overall recovery of the breakdown products was lower, compared with the corresponding profile in the broccoli sprouts, especially when treated at 100 °C. Nitriles are comparatively stable substances and need forceful conditions like high temperatures or strong acidic or basic medium to be hydrolyzed to carboxylic acids.[37] To test the stability of nitriles under the conditions applied for the broccoli sprouts, the model compounds phenyl-CN and 3-butenyl-CN were treated similarly. As expected, only a slight degradation was observed. Therefore, diminutions of the nitrile concentrations while roasting broccoli sprouts should be resulting from reactions with the surrounding plant matrix. In contrast to the nitriles, isothiocyanates are thought to be thermolabile and degrade in aqueous solution, even at physiological temperature(s), to form volatile and nonvolatile compounds.[31,38] For example, 62% of allyl isothiocyanate are degraded at pH 2.7 after 1 h of boiling. Basic conditions (pH 9) lead to an enhanced degradation.[38] The nonvolatile *N,N'*-dialkyl thiourea results from the breakdown of sulforaphane and allyl isothiocyanate and especially allyl isothiocyanate forms volatile substances with a garlic smell.[38,39] Therefore, low recovery rates of thermal breakdown products, especially in the experiments with the isolated GSL mixture, could also be due to a higher ratio of isothiocyanate formation. But, because of their further degradation, nitriles remain the main breakdown products.

In summary, the present study reveals that sulfur-containing aliphatic GSL in broccoli sprouts show a structure-dependent thermally induced degradation, resulting in the primarily formation of nitriles. To our knowledge this is the first study that monitored the formation of thermally induced breakdown products of sulfur-containing aliphatic GSL depending on different influencing factors in a *Brassica* vegetable (matrix). Methylsulfanylalkyl GSL were more susceptible to degradation at high temperatures, whereas methylsulfinylalkyl GSL were revealed to be more affected by aqueous basic conditions. The reasons for these differences in the stability of individual sulfur-containing aliphatic GSL remain still unclear and will be subject of further studies. Whereas nitriles were rapidly formed at the dry heat conditions, the highest concentrations were found during cooking in acidic medium. At a first glance, one can conclude that higher temperatures favor GSL degradation, but the formation of breakdown products is not in a linear relationship, as further reactions with other constituents of the plant matrix, such as proteins, might occur.[40] There also seem to be differences in the stability/reactivity of the nitriles. As the recovery rates of the methylsulfinylalkylnitriles are comparatively low, they seem to be more reactive compared to the methylsulfanylalkylnitriles.

On the other hand, isolated GSL differed in their degradation behavior when compared to GSL in the plant matrix. Generally, the sulfur-containing aliphatic GSL are more stable compared to the situation in broccoli. It was also observed that structural differences in the thermally induced degradation were not present anymore. Therefore, the matrix has a great impact on the thermally induced degradation of GSL. It was demonstrated that iron favors thermally induced GSL breakdown and nitrile formation. The chemical mechanism of the thermally induced degradation of GSL will be the focus of further studies.

Thermally induced degradation of health-beneficial sulfur-containing aliphatic GSL will result in reducing the health benefits of the vegetables as potentially toxic nitriles are formed and only lower concentrations of preventive isothiocyanates are released. Thence, it seems to be advantageous that thermal treatment of *Brassica* vegetables should be minimized to preserve the health-beneficial effects of these foods.

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Funding

This work was financially supported by the German Federal Ministry of Education and Research (BMBF) through the Project Management Jülich, Grant No. FKZ: 0315370B.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

Mrs. Conny Richter is thanked for excellent technical assistance.

ABBREVIATIONS USED

3-butenyl-CN, 4-pentenenitrile; CN, nitrile; GSL, glucosinolate; 3-MTP, 3-(methylsulfanyl)propyl GSL; 3-MSOP, 3-(methylsulfinyl)propyl GSL; 4-MTB, 4-(methylsulfanyl)butyl GSL; 4-MSOB, 4-(methylsulfinyl)butyl GSL; 5-MSOP, 5-(methylsulfinyl)pentyl GSL; 3-MTP-CN, 4-methylsulfanylbutylnitrile; 3-MSOP-CN, 4-methylsulfinylbutylnitrile; 4-MTB-CN, 5-methylsulfanylpentylnitrile; 4-MSOB-CN, 5-methylsulfinylpentylnitrile; phenyl-CN, benzonitrile.

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