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***ACCEPTED MANUSCRIPT***

# Domestic boiling and salad preparation habits affect glucosinolate degradation in red cabbage (*Brassica oleracea* var. *capitata* f. *rubra*)

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## Abstract

Red cabbage contains glucosinolates, precursors to health-promoting isothiocyanates. However, raw cabbage often releases mainly epithionitriles and nitriles from glucosinolates. To increase isothiocyanate formation, the effect of acid usage in the preparation of red cabbage was evaluated. Moreover, the effects of the chosen boiling method (acidic boiled red cabbage versus neutral boiled blue cabbage) on glucosinolate degradation were investigated using UHPLC-DAD-ToF-MS and GC-MS. The addition of vinegar significantly increased isothiocyanate formation of cabbage salad from 0.09 to 0.21 µmol/ g fresh weight, while lemon juice only slightly increased isothiocyanate formation. Acidic boiled red cabbage degraded glucosinolates and increased nitrile formation, while in neutral boiled blue cabbage, glucosinolates were stable. However, shortly boiled blue cabbage (5 min) had the highest isothiocyanate levels (0.08 µmol/ g fresh weight). Thus, for a diet rich in isothiocyanates it is recommended to acidify raw cabbage salads and prepare shortly boiled blue cabbage instead of red cabbage.

Keywords: Glucosinolates, Isothiocyanates, Epithionitriles, *Brassica*, Processing, Nitriles, pH, Blue cabbage

## 1. Introduction

Red cabbage (*Brassica oleracea* var. *capitata* f. *rubra*) accounts for 14% of all produced cabbages in Germany (2018) and is part of many traditional dishes, such as in salad or as a boiled vegetable dish, as well as in modern ones like the doner kebab. This vegetable contains glucosinolates (GLSs), secondary plant metabolites that have positive effects on human health. Of note is that consumption of GLS-rich *Brassica* vegetables is linked with reduced incidences of developing certain types of cancer (Kolonel, Hankin, Whittemore, Wu, Gallagher, Wilkens, et al., 2000; Terry, Wolk, Persson, & Magnusson, 2001; Veeranki, Bhattacharya, Tang, Marshall, & Zhang, 2015). These effects are attributed to isothiocyanates (ITCs), which are electrophilic and reactive GLS hydrolysis products (Palliyaguru, Yuan, Kensler, & Fahey, 2018). Especially 4-(methylsulfinyl)butyl ITC (4MSOB-ITC, sulforaphane), released from broccoli and red cabbage (Hanschen & Schreiner, 2017), is valued for its strong chemopreventive properties (Palliyaguru, Yuan, Kensler, & Fahey, 2018). When plant tissue is damaged, GLSs encounter myrosinase, a *β*-D-thioglucosidase, which cleaves glucose to release a thiohydroximate-*O*-sulfate. This aglucon spontaneously rearranges to the ITC or to a nitrile and sulfur (**Figure 1**). Moreover, in *Brassica oleracea*, three epithiospecifier protein (ESP) isomers rearrange alkenyl-aglucons to form epithionitriles (ETNs) (Witzel, Abu Risha, Albers, Börnke, & Hanschen, 2019). ESPs can also enhance the formation of nitriles from saturated aglucons, and therefore, inhibit the formation of cancer preventive 4MSOB-ITC in broccoli (Matusheski, Swarup, Juvik, Mithen, Bennett, & Jeffery, 2006). Thus, many *Brassica oleracea* vegetables release high amounts of ETNs and nitriles from the GLSs instead of health-promoting ITCs (Hanschen & Schreiner, 2017; Kyung, Fleming, Young, & Haney, 1995). Since ETNs and nitriles are considered to have lower health-beneficial potential compared to ITCs (Hanschen, Herz, Schlotz, Kupke, Bartolomé Rodríguez, Schreiner, et al., 2015; Matusheski & Jeffery, 2001), it is desirable to enhance ITC formation in foods. Red cabbage typically is consumed raw as salad or as a boiled vegetable dish. These processing techniques severely affect the GLS regime. In detail, during cutting, some of the GLSs enzymatically degrade releasing ITCs, nitriles and ETNs. Hydrolysis conditions, such as

52 temperature and pH value, can affect the ratios of these hydrolysis products and a more acidic
53 or more basic pH value can also lead to enhanced ITC formation (Hanschen, Klopsch, Oliviero,
54 Schreiner, Verkerk, & Dekker, 2017). Thus, it was hypothesized that adding acids, such as
55 vinegar or lemon juice, during salad preparation would promote ITC formation (Hanschen,
56 Klopsch, Oliviero, Schreiner, Verkerk, & Dekker, 2017). Moreover, during cooking, hydrolyzing
57 enzymes are degraded with ESPs being already inactivated when temperatures reach 60°C.
58 Thus, ITC formation in vegetables can be increased by short thermal treatment (Matusheski,
59 Juvik, & Jeffery, 2004). However, during boiling, GLS levels in vegetables decline due to
60 leaching (Sarvan, Verkerk, & Dekker, 2012; Volden, Borge, Hansen, Wicklund, & Bengtsson,
61 2009). Finally, GLS can be thermally degraded (Oerlemans, Barrett, Suades, Verkerk, &
62 Dekker, 2006) and nitriles can be formed when heating in closed systems (Hanschen, Kühn,
63 Nickel, Rohn, & Dekker, 2018; Hanschen, Platz, Mewis, Schreiner, Rohn, & Kroh, 2012). To
64 date, cooking studies using open systems like conventional boiling on the formation of GLS
65 degradation products are rare and often only ITCs are considered (Baenas, Marhuenda,
66 García-Viguera, Zafrilla, & Moreno, 2019). One study on boiling Brussels sprouts found mainly
67 GLS-derived nitriles that increased with boiling time (Ciska, Drabińska, Honke, & Narwojsz,
68 2015). In contrast, steaming and boiling pak choi was found to severely decrease ETN and
69 nitrile levels, so ITCs were the prevailing degradation products of cooked pak choi (Chen,
70 Hanschen, Neugart, Schreiner, Vargas, Gutschmann, et al., 2019).
71 Thus, to the best of knowledge, this is the first study to investigate how different domestic
72 preparation techniques with different additives, such as acids, affect the formation of GLS
73 degradation products. Importantly, to provide valuable dietary recommendations to the
74 consumer to increase the benefit from GLS-rich *Brassica* vegetables, it is essential to know
75 the effect of usual domestic preparation techniques. Here, the hypothesis is tested, how
76 acidification using typical additives for salad preparation, i.e. vinegar or lemon juice, will
77 enhance ITC levels during red cabbage salad preparation. Further, the effect of domestic-like
78 preparation techniques, including the addition of vinegar or lemon juice ("red cabbage"), or
79 $NaHCO_3$ („blue cabbage"), during boiling of red cabbage on GLSs and degradation products

is evaluated. This study aims to provide valuable and practical consumer advice on how to prepare *Brassica oleracea* vegetables with enhanced formation of health-promoting ITCs.

## 2. Methods

### 2.1 Chemicals and enzymes

4-hydroxybenzyl GLS (≥ 99%), methylene chloride (GC Ultra Grade) and 2-propenyl GLS were purchased from Carl Roth GmbH + Co. KG (Karlsruhe, Germany); allyl ITC (≥ 99%), aryl sulfatase, benzonitrile (≥ 99.9%), 3-butenenitrile (≥ 98%), DEAE-Sephadex A-25, 4-pentenenitrile (≥ 97%), and 3-phenylpropanenitrile (≥ 99%) were obtained from Sigma-Aldrich Chemie GmbH (Steinheim, Germany); 3-butenyl ITC (≥ 95 %) and 4-pentenyl ITC (≥ 95%) were purchased from TCI Deutschland GmbH (Eschborn, Germany); $NaSO_4$ anhydrous (≥ 99%) was obtained from VWR International GmbH (Darmstadt, Germany); $NaHCO_3$ (p.A, ≥ 99%) was purchased from Merck (Darmstadt, Germany); 3-(methylsulfinyl)propyl ITC and 4-(methylsulfanyl)butyl ITC (≥ 98 %) were purchased from Santa Cruz Biotechnology (Heidelberg, Germany); 4MSOB-ITC was purchased from Enzo Life Sciences GmbH (Lörrach, Germany). The ETN 1-cyano-2,3-epithiopropane (≥ 95 %) was synthetized by Taros Chemicals GmbH Co. KG (Dortmund, Germany) and 1-cyano-3,4-epithiobutane was synthetized by ASCA GmbH Angewandte Synthesechemie Adlershof (Berlin, Germany). 5-(Methylsulfanyl)pentanenitrile and 5-(methylsulfinylpentanenitrile were purchased from Enamine (SIA Enamine, Latvia, Riga). Methanol (≥ 99.95 %), acetonitrile (LC-MS grade), and arylsulfatase were purchased from Th. Geyer GmbH & Co. KG (Renningen, Germany) and ultrapure water was used.

### 2.2 Plant material

Seeds of red cabbage *B. oleracea* var. *capitata* f. *rubra* cv. Integro (Bejo Samen GmbH, Sonsbeck, Germany) were sown in substrate (Einheitserde Classic P, Einheitserde Werkverband e.V., Germany) and grown in an open-sided greenhouse at the Leibniz Institute of Vegetable and Ornamental Crops in Grossbeeren, Germany. After 18 days, plants were

transplanted into seed growing trays filled with substrate (Einheitserde Classic T, Einheitserde Werkverband e.V., Germany). When plants were 2 months old, they were transferred outside into two randomly selected plots of an open-air facility site, established in Grossbeeren (52°20'58.4''N 13°18'52.9''E) in 1972, with sandy soil filled-in concrete plots (2 m x 2 m base area, 0.75 m depth). The plots were 4 $m^2$ each and contained 16 plants with a row/plant distance of 50/50 cm. Until harvest, plants were protected from insects by cultivation nets to prevent plants from being affected by pathogens or insects. According to standard practice, fertilization prior to planting and 1 month after planting was given by 200 g calcium ammonium nitrate/plot. Water was provided as needed throughout the growth period. For the experiments that were performed in 4 days in autumn 2018, the fully developed heads were harvested freshly.

**2.3 Experimental design**

The effect of acid addition during preparation of red cabbage salad and the effect off the addition of acid or $NaHCO_3$ during boiling red (acidic) or blue (neutral/basic) cabbage on GLS levels and the formation of their breakdown products was evaluated.

For each experiment, three or four freshly harvested red cabbage heads (from the two plots) were harvested. The outer leaves were discarded. The red cabbages were then cut into small uniform pieces (approx. 3 x 4 x 2 mm), producing a homogeneous mixed sample for each of the three to four cabbage heads. From these vegetable pieces, 50 g were weighed into a 150 mL glass beaker. On the first experimental day (101 days after sowing), the effect of different acids for salad preparation was evaluated: To the 50 g of finely chopped red cabbage, either 2.5 mL of 10% brandy vinegar (Bautz'ner Branntwein Essig 10% Säure, Bautz'ner Senf & Feinkost GmbH, Bautzen, Germany) or 2.5 mL of lemon juice (Fruchtstern Zitronensaft 100% Fruchtgehalt, Netto Marken-Discount AG & Co. KG, Maxhütte-Haidhof, Germany) were added. For the control, 2.5 mL of water was used. The cabbage was then mixed with the respective liquid using a glass bar, the beaker was closed with a watch glass, and the "salad" was left at room temperature for 1 hour. Four independent replicates (using material form four cabbages)

were performed for each treatment. After which, the salad was thoroughly mixed and samples were taken for GLS analysis: 3 g of the cabbage salad were weighed into a 20 mL Polyvial® (Zinsser Analytic GmbH, Frankfurt, Germany), frozen in liquid nitrogen, freeze dried, and ground to a fine powder, which was stored at room temperature until analysis. For GLS degradation product analysis, 4 g of cabbage salad mixed with 4 g of water were homogenized in a 20 mL Polyvial® (Zinsser Analytic GmbH, Frankfurt, Germany) using a mixer mill and a frequency of 30 Hz (Retsch MM 400, Retsch GmbH, Haan, Germany). From this, 1 gram of the homogenized plant material was then used for GLS degradation product analysis.

On the three other days the boiling experiments were performed. On the second (104 days after sowing) and third (105 days after sowing) days of experiments, the influence of vinegar or lemon juice addition for red cabbage cooking was evaluated. On the fourth (119 days after sowing) day of experiments; the effect of soda addition for cooking blue cabbage was assessed. For this purpose, 50 g cabbage in the beaker was placed on the heating plate. After 45 seconds, 50 mL boiling water were added. Then, either 2.5 mL of the vinegar, lemon juice, or of a 20 g/L (0.238 M) $NaHCO_3$ solution (2.5 mL contained 50 mg of $NaHCO_3$; the value was experimentally determined to be sufficient to color the cabbage blue) were added. The cabbage was stirred, a watch glass was added, and samples were cooked for 0 min [non-heated water was used, sample was not heated], 5 min, 10 min, 20 min, 30 min, and 60 min on a heating plate set to heating level 5-7 (Stuart Hotplate CB 500, Cole-Parmer, United Kingdom). The water level was kept constant by adding boiling water if necessary. Three independent replicates (using material form three different cabbages) were performed for each boiling time.

Afterwards, samples were immediately cooled using an ice water bath and taken for GLS analysis by weighing 3 g (containing cooking water and cabbage material in equal amounts) into a 20 mL Polyvial®. Samples were prepared as described above. For GLS degradation product analysis, 8 g cooked cabbage material (including cooking water) were homogenized as described above. From this, an aliquot (4 g) was used for GLS hydrolysis product analysis.

164 **2.4 Analysis of GLSs as desulfo-GLSs by UHPLC-DAD-ToF-MS**

165 For the GLSs analysis, the DIN EN ISO 9167-1 based method as described previously
166 (Wiesner, Zrenner, Krumbein, Glatt, & Schreiner, 2013) was applied with some modifications.
167 Briefly, 10 mg of lyophilized and ground plant tissue was extracted 3-times using 70% methanol
168 in the presence of 0.025 µmol 4-hydroxybenzyl GLS as an internal standard. The combined
169 extracts were desulfated using aryl sulfatase and DEAE-Sephadex A-25 ion-exchanger
170 columns and then eluted with 1 mL of water as described previously (Wiesner, Zrenner,
171 Krumbein, Glatt, & Schreiner, 2013). After which, 5 µL were injected into an 1290 Infinity II
172 ultra-high performance liquid chromatography with a diode array detection (UHPLC-DAD)
173 system coupled with a 6230 liquid chromatography time-of-flight mass spectrometry (LC-ToF-
174 MS) (Agilent Technologies, Waldbronn, Germany) equipped with a Poroshell 120 EC-C18
175 column (100 mm x 2.1 mm, 2.7 µm; Agilent Technologies) as described previously (Klopsch,
176 Witzel, Börner, Schreiner, & Hanschen, 2017). Analytes were separated at 30 °C with a flow
177 rate of 0.4 mL $min^{-1}$ using a gradient of water (A) and 40% acetonitrile (B) starting at 0.5% B
178 (2 min hold), rising to 49.5% B within 10 min (2 min hold), increasing within 1 min to 99.5% B
179 (2 min hold). MS parameters were as follows: Multimode source settings: positive mode, 300°C
180 gas temperature, 200°C vaporizer temperature, 8 L/min gas flow, VCharge 2000V, nebulizer
181 35 psig, VCap 2500V, CoronaPositive 4µA; MS TOF settings: Fragmentor 175V, Skimmer 1
182 65V, OctopoleRFPeak 750V, scan range 100-1700m/z. Desulfo-GLSs were identified by
183 comparing retention times, UV absorption spectra, and mass spectra with those of individual
184 desulfo-GLSs from standard reference materials of oilseed rape (BCR-190R and BCR-367R)
185 and with analytical standards. GLSs were quantified at 229 nm via the internal standard and
186 the response factor (RF) reported in the DIN EN ISO 9167-1 and calculated on this basis for
187 4-hydroxybenzyl GLS.

188

189 **2.5 Determination of GLS breakdown products by GC-MS**

190 GLS breakdown products were extracted from 1 g (“cabbage salad”) or 4 g (boiled sample) of
191 the homogenized plant material (containing 50% of water) or from 3 g of non-homogenized

192 “cabbage salad”. The sample was placed in a solvent-resistant vessel, extracted, and analyzed
193 as previously reported (Hanschen & Schreiner, 2017) using a transfer line temperature of the
194 gas chromatography-mass spectrometry (GC-MS) system of 270°C. Briefly, GLS breakdown
195 products were extracted two times with 2 mL of methylene chloride in the presence of the
196 internal standard (0.2 µmol benzonitrile), extracts were dried with anhydrous $NaSO_4$,
197 concentrated to 300 µL under nitrogen gas flow, and transferred into a vial. Samples were
198 analyzed using the Agilent 7890A Series GC-MS System (mass selective detector (MSD):
199 5975C inert XL) (Agilent Technologies, Waldbronn, Germany), a SGE BPX5 column (30 m×
200 0.25 mm× 0.25 µM) (VWR International GmbH, Darmstadt, Germany), and the GC conditions
201 reported previously (Franziska S. Hanschen & Schreiner, 2017) with the transfer line set to
202 270°C.
203
204 **2.6 Determination of pH values by a pH meter**
205 The pH values of the cabbage samples were measured after the treatments using a 691 pH
206 Meter (Metrohm AG, Herisau, Switzerland). In the case of the raw cabbage salads, the pH
207 value measurements were taken for the non-homogenized material as well as after
208 homogenization. In the case of boiled samples, the pH value of the watery phase (no
209 homogenization) was measured. All samples had an ambient temperature.
210
211 **2.7 Analysis of the acid content of vinegar and lemon juice by titration**
212 The acid content was determined by diluting 1 mL vinegar or lemon juice with 49 mL of water.
213 The diluted acid was then titrated using a TitroLine easy titrator (SCHOTT-GERÄTE GmbH,
214 Mainz, Germany) until pH 8.1 using a 0.1 N NaOH solution (Titrisol®, Merck KGaA, Darmstadt,
215 Germany) (Amtl. Sammlung von Untersuchungsverfahren nach §35 LMBG, L26.11.03-4,
216 1983). The acid content was analyzed in duplicate.
217
218 **2.8 Statistical analysis**

To investigate differences between different treatments, means were compared using the ANOVA and Tukey's HSD test and STATISTICA version 13.2 software (StatSoft, Hamburg, Germany) with a significance level of $p \leq 0.05$. All experiments were carried out in triplicate (boiling experiments) or quadruplicate ("cabbage salad").

## 3. Results

The effect of different domestic-like preparation techniques of red cabbage on GLS content and the formation of their degradation products was evaluated. Different additives in the preparation of raw red cabbage salad and for boiled red cabbage were compared.

### 3.1 Characterization of acid content in domestic acids

The pH values measured for the vinegar and lemon juice were 3.1 and 2.9, respectively. In the case of vinegar 1 mL consumed 17.21 ± 0.04 mL of 0.1 N NaOH and in the case of lemon juice, 1 mL needed 8.16 ± 0.08 mL of 0.1 N NaOH until reaching pH 8.1. Thus, the acid content was calculated to be 103 ± 0.2 mg/mL acetic acid for the vinegar and 52.2 ± 0.5 mg/mL citric acid for the lemon juice.

### 3.2 Effect of acidification during red cabbage salad preparation

#### 3.2.1 Effect of acid addition on pH values of red cabbage salad

In the first experiment, the effect of adding vinegar or lemon juice during red cabbage salad preparation on the formation of GLS hydrolysis products was evaluated and compared to a water control. Adding vinegar or lemon juice to the cabbage salad, significantly reduced the pH value from pH 6.4 to pH 4.2 and pH 4.5, respectively. These pH values were measured in the non-homogenized salad (**Table 1**). However, the pH values of homogenized and non-homogenized samples were equivalent (**Table 1**).

#### 3.2.2 Effect of acid addition on GLSs and their hydrolysis products in red cabbage salad

The GLSs present in the red cabbage salad samples were analyzed. The main GLSs in the control salad were indol-3-ylmethyl GLS [0.163 ± 0.023 µmol/g fresh weight (FW)] and 4-

(methylsulfinyl)butyl GLS (0.144 ± 0.005 µmol/g FW). Other abundant GLSs were 3-(methylsulfinyl)propyl GLS (0.067 ± 0.004 µmol/g FW), the alkenyl GLSs allyl GLS (0.033 ± 0.004 µmol/g FW), 3-butenyl GLS (0.061 ± 0.007 µmol/g FW), and 2-(*R*)-2-hydroxy-3-butenyl GLS (0.080 ± 0.016 µmol/g FW). Accordingly, the acidic treatments reduced the total alk(en)yl GLS content (22% for vinegar and 15% for lemon juice). However, this reduction was not statistically significant (**Figure 2A**).

With regard to the GLS hydrolysis products analyzed in the red cabbage salad, the main GLS hydrolysis products detected in the samples were 4MSOB-ITC (sulforaphane) and its corresponding nitrile, whereas products from the indole GLS were found only in very low amounts. In the red cabbage salad samples, GLS hydrolysis products were analyzed before and after homogenization, to test which hydrolysis products were already there after incubating the salad for 1 h. Without the homogenization step, more nitriles and ETNs were found in the "water control salad" and more ITC were found in the non-homogenized vinegar salad compared to the homogenized samples (**Figure 2A**). Thus, homogenization did not further increase levels of GLS hydrolysis products. Adding vinegar to the salad more than doubled ITC levels and reduced ETN levels compared to the water control. While the addition of lemon juice increased the ITC levels (but not significantly) it did decrease the ETN levels significantly (80% reduction; $p \leq 0.05$, Tukey's HSD-test) in the non-homogenized material compared to the "water control salad" (**Figure 2A**). When comparing the ratio of ITC formation, there was no difference between non-homogenized and homogenized material. However, both acidic treatments significantly ($p \leq 0.05$, Tukey's HSD-test) increased ITC ratios. In detail, vinegar increased the ITC ratio by 81-110%, while lemon juice addition increased the ITC ratio by 30-65% compared to the "water control salad" (**Figure 2B**). Especially in the vinegar salad, the ratio of ETNs and nitriles decreased by 90% and 47-65%, respectively, compared to the "water control salad".

### 3.3 Influence of boiling on GLSs and breakdown products in red and blue cabbage

#### 3.3.1 Effect of boiling treatments on pH values of red cabbage

275 Red cabbage was prepared as a “red cabbage” by adding vinegar or lemon juice or as a “blue
276 cabbage” by adding $NaHCO_3$ during boiling. The three different treatments differently affected
277 the pH value of the boiled cabbage (**Table 1**). Addition of vinegar resulted in the most acidic
278 pH range (pH 4.2-4.4), while lemon juice reduced the pH of the cabbage less strongly (pH 4.3-
279 4.5). Addition of $NaHCO_3$ increased the “blue cabbage” pH value to a neutral pH value (pH
280 6.6-7.2). During cooking, the pH within the three boiling treatments slightly changed: in the
281 acidic treatments, it was lowest at the beginning and increased with cooking time (maximum
282 cooking time 60 min). In contrast, in the $NaHCO_3$ treatment, pH increased until 10-20 min of
283 cooking and then decreased, so that after 60 min the pH value was lowest (**Table 1**).

284 **3.3.2 Effect of boiling treatments on GLS and degradation products in red cabbage**

285 The GLSs present in the cooked samples were the same as detected in the red cabbage salad,
286 but absolute values were lower due to the dilution with the boiling water. In the experiment with
287 the vinegar addition, the GLS samples thawed before they were freeze dried. Therefore, the
288 data especially of the non-heated sample will not be considered here. In red cabbage boiled
289 with lemon juice, total alk(en)yl GLS decreased with cooking time (**Figure 3B**) and were
290 reduced to 57% after 60 min compared to the non-heated sample. In blue cabbage boiled with
291 $NaHCO_3$, the total alk(en)yl GLS were not changed with boiling time (**Figure 3C**). The levels
292 of the individual GLSs in the lemon juice- and $NaHCO_3$-treated boiled cabbage are presented
293 in **Supplemental Figure 1**. The indole GLS 4-hydroxyindol-3-ylmethyl GLS was the least
294 stable and methylsulfanylalkyl GLSs were by tendency less stable compared to the
295 methylsulfinylalkyl and alkenyl GLSs.

296 The GLS breakdown products detected in the boiled red cabbage samples changed with the
297 cooking time. In the red cabbage cooked with vinegar, ITCs, which were the main products in
298 the unheated sample, decreased by 93% within the first 10 min and then stayed at that level
299 throughout the boiling process (**Figure 3A**). Nitriles increased with cooking times longer than
300 10 min, so that they were the main GLS degradation products in samples boiled for 10 min or
301 more: After 60 min, nitriles made up 84% of all GLS degradation products and their
302 concentration increased by 229% compared to the non-heated control. ETNs decreased by

303 74% in the first 5 min of cooking and then stayed at that level. In the red cabbage cooked with
304 lemon juice, a similar trend for GLS breakdown products was found (**Figure 3B**). ITCs and
305 ETNs decreased by 76% and 96% within the first 5 min of cooking and stayed at that level.
306 Nitriles, however, decreased by 85% within the first 5 min of cooking, but then increased with
307 further cooking time, so that after 60 min there were 7-times more nitriles compared to the 5
308 min boiled samples (**Figure 3B**). When blue cabbage was cooked by adding $NaHCO_3$ to the
309 red cabbage, a different behavior in GLS hydrolysis product formation was observed compared
310 to the acidic treatments. Within the first 5 min of boiling, the ITCs content doubled (but effect
311 was not significant), so that in the 5 min boiled samples, the highest ITC level was present.
312 Then, the ITC decreased with further cooking time and after 60 min of boiling only 44% of non-
313 heated controls were left (**Figure 3C**). Like nitriles, the ETNs decreased within the first 5 min
314 of cooking by 91% (nitriles by 83%) and stayed at that level over the entire cooking period
315 (**Figure 3C**).
316

317 ## 4. Discussion

318 Red cabbage can be prepared in very different ways, which will affect the presence of GLS
319 and the formation of ITC and other GLS degradation products. Here, the hypothesis was tested
320 whether the addition of domestic acids in the preparation of cabbage salad will increase ITC
321 levels. For acidic additives vinegar as well as lemon juice were evaluated. Moreover, the effect
322 of different domestic preparation techniques of boiled red or blue cabbage on the GLS content
323 and degradation products was tested.
324 The red cabbage was rich in 4-(methylsulfinyl)butyl GLS as well as the indole GLS indol-3-
325 ylmethyl GLS, and thus, had a similar GLS profile compared to previous reports (Ciska,
326 Martyniak-Przybyszewska, & Kozłowska, 2000; Hanschen & Schreiner, 2017; Oerlemans,
327 Barrett, Suades, Verkerk, & Dekker, 2006). From 4-(methylsulfinyl)butyl GLS upon hydrolysis
328 in the “water control salad”, both the nitrile and corresponding 4MSOB-ITC (sulforaphane)
329 were released in a ratio 1:1 (in the non-homogenized sample). Thus, in the control more ITCs
330 were formed compared to a previous study on *Brassica rapa*, *Brassica oleracea*, and

*Arabidopsis thaliana* (Hanschen, Klopsch, Oliviero, Schreiner, Verkerk, & Dekker, 2017). This was probably due to lower ESP activity since these proteins play a role in enzymatic nitrile formation in *Brassica* vegetables (Matusheski, Swarup, Juvik, Mithen, Bennett, & Jeffery, 2006).

In red cabbage salads prepared with vinegar or lemon juice as well as the water control, the GLS content of the three different salad samples did not differ significantly, thereby suggesting that the treatments did not inhibit or accelerate the enzymatic hydrolysis of the GLSs. However, the acetic treatments affected the formation and ratios of GLS hydrolysis products and the acid addition increased ITC levels, which has been hypothesized previously by our group (Hanschen, Klopsch, Oliviero, Schreiner, Verkerk, & Dekker, 2017). While the effect of lemon juice only resulted in a slight increase of ITC levels, vinegar addition doubled the levels in the red cabbage salad (**Figure 2A and 2B**). This finding is probably due to the higher acid content of the vinegar used, which was also reflected in the lower pH value of the vinegar salad samples compared to the lemon juice ones (**Table 1**). The increased ITC formation was at the expense of nitrile and ETN release. Therefore, reducing the pH value with acids probably reduces the activity of ESP, which in *Brassica napus* and *Crambe abyssinica*, has the optimal pH value at pH 6 (Bernardi, Negri, Ronchi, & Palmieri, 2000; Tookey, 1973). Recently, recombinant *Brassica oleracea* ESPs were shown to be differently affected by reduced pH values and BoESP2 at pH 4 had only 27% of its activity compared to pH 6 (Witzel, Abu Risha, Albers, Börnke, & Hanschen, 2019).However, also an increase in myrosinase activity could explain higher ITC formation since the degradation of the thiohydroximate-*O*-sulfate, which is considered to be the substrate of ESP, to the ITC is spontaneous (Backenköhler, Eisenschmidt, Schneegans, Strieker, Brandt, & Wittstock, 2018). Moreover, the pH optimum of *Arabidopsis thaliana* myrosinases TGG1, TGG4, and TGG5 is known to be optimal in a wide pH range of pH 5.5 to 10.5, but slightly reduced at pH 4.5, and even more strongly reduced at pH 3.5 (Andersson, Chakrabarty, Bejai, Zhang, Rask, & Meijer, 2009). In addition, Brussels sprouts (*Brassica oleracea* var. *gemmifera*) myrosinase has high activities between pH 6 and pH 8.5, whereas this activity is decreased by approximately 70% at pH 4.5 (Springett & Adams,

359 1989). Thus, since the GLS content of the acidic salads were not significantly lower, it is
360 assumed that myrosinase activity did not increase, but probably decreased due to acid
361 addition.
362 GLS hydrolysis occurs when plant tissue is disrupted and GLS and myrosinase come into
363 contact. To determine whether cutting cabbage is already sufficient to release GLS in
364 significant amounts, the salads were analyzed with and without an additional homogenization
365 step, which was predicted to further promote hydrolysis reaction. When comparing the levels
366 of GLS hydrolysis products of the salad analyzed before or after a homogenization step, the
367 homogenization did not lead to a further release of hydrolysis products. In some cases (water
368 control), even lower GLS hydrolysis product levels where found in the homogenized samples,
369 although GLS were still present. It is suspected that myrosinase activity decreased after cutting
370 and during incubation of the salad samples, so that the late homogenization step did not
371 contribute to further breakdown product formation. As a result, manual cutting seems to be
372 sufficient to cause a high GLS hydrolysis product formation – although GLS hydrolysis due to
373 manual cutting was not entirely complete. However, ingested intact GLSs can be further
374 metabolized by the gut microbiota. Here, ITC formation is strongly dependent on the strains
375 present (Liou, Sirk, Diaz, Klein, Fischer, Higginbottom, et al., 2020).
376 With regard to the red cabbage cooked with lemon juice (pH 4.3-4.4), GLSs were less stable
377 compared to the blue cabbage cooked with $NaHCO_3$ (pH 6.6-7.2). Of interest is that Gronowitz
378 et al. reported that *R*-2-hydroxy-3-butenyl GLS when cooked in buffers was most stable at pH
379 7 compared to higher and lower pH values (Gronowitz, Svensson, & Ohlson, 1978). However,
380 in a more recent study, no difference in GLS stability was found between broccoli sprouts
381 powder boiled at pH 4.8 and pH 7, except for 3-(methylsulfanyl)propyl GLS, which was more
382 stable at pH 7 (Hanschen, Rohn, Mewis, Schreiner, & Kroh, 2012). Here, the stability of
383 individual GLSs (**Supplemental Figure 1**) was similar to previous reports (Hanschen, Rohn,
384 Mewis, Schreiner, & Kroh, 2012; Oerlemans, Barrett, Suades, Verkerk, & Dekker, 2006).
385 Boiling the red cabbage under acetic conditions reduced ITC and ETN formation drastically.
386 Such a reduction can be linked to either further chemical degradation since these compounds

are not stable or a decline due to evaporation. For example, when heated under aqueous conditions at 100°C, the ETN 1-cyano-2,3-epithiopropane had a half-life period of 68 min at pH 5 compared to only 6 min at pH 7 and 2-aminothiophene was identified as the main product (Hanschen, Kaufmann, Kupke, Hackl, Kroh, Rohn, et al., 2018). Moreover, 4MSOB-ITC is also instable and at 100°C had a half-life of 56 min at pH 5 (Fechner, Kaufmann, Herz, Eisenschmidt, Lamy, Kroh, et al., 2018).

Nevertheless, in acetic boiled red cabbage, nitriles increased with longer boiling times, probably due to thermal degradation of GLSs. While thermal degradation of GLSs in boiled *Brassica* vegetables favors the formation of nitriles (Ciska, Drabińska, Honke, & Narwojsz, 2015; Hanschen, Kühn, Nickel, Rohn, & Dekker, 2018; Hanschen, Platz, Mewis, Schreiner, Rohn, & Kroh, 2012), thermal degradation of isolated GLSs can also favor ITC formation or products that derive from ITC such as oxazolidine-2-thiones (Gronowitz, Svensson, & Ohlson, 1978; Hanschen, Bauer, Mewis, Keil, Schreiner, Rohn, et al., 2012). Notably, Ciska et al. (2015) also observed an increase in especially 3-butenenitrile and indole-3-acetonitrile levels when boiling Brussels sprouts in an open system (Ciska, Drabińska, Honke, and Narwojsz (2015). In the present study, boiling blue cabbage under neutral conditions was not accompanied with nitrile formation and GLS degradation products decreased with cooking time. This observation is probably due to the fact that GLSs were quite stable, and thus, the levels of degradation products declined faster due to volatilization than they were formed due to thermal GLS degradation. In models with boiling broccoli sprouts (100°C), the GLS were more labile at pH 8 compared to pH 5.3; however, more nitriles were formed at pH 5.3 compared to pH 8 (Hanschen, Platz, Mewis, Schreiner, Rohn, & Kroh, 2012). In contrast, in models of pure allyl GLS heated aqueously at 100°C, the ratio of nitrile to ITC levels increased at pH 8 versus pH 5.3 (Hanschen, et al., 2012). Interestingly, the blue cabbage cooked for 5 min had the highest ITC levels among the cooked samples, thus indicating that ESP activity was reduced due to the treatment, but myrosinase probably was still active under these neutral cooking conditions. Therefore, cooking with $NaHCO_3$ for short times can be a strategy to increase ITC release in red cabbage.

## 5. Conclusion

In the present study, the effect of domestic-like preparation techniques of red cabbage on GLSs and the formation of their degradation products was evaluated. With regard to ITC release, salad preparation with the addition of acid can be recommended, especially with vinegar, since this treatment doubled ITC levels. Moreover, boiling acid-treated red cabbage leads to higher nitrile formation due to thermal GLS degradation, while blue cabbage preparation with alkaline $NaHCO_3$ can strongly increase ITC levels if boiled for only 5 min. Thus, to the best of knowledge, this study provides for the first time basic consumer advice on how to prepare *Brassica oleracea* var. *capitata* f. *rubra* in order to obtain optimal levels of the desired health-promoting ITCs.

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### Conflict of Interest

There author declares no conflict of interest.

## References

Amtl. Sammlung von Untersuchungsverfahren nach §35 LMBG, L26.11.03-4. (1983).

441 Andersson, D., Chakrabarty, R., Bejai, S., Zhang, J., Rask, L., & Meijer, J. (2009). Myrosinases
442 from root and leaves of *Arabidopsis thaliana* have different catalytic properties.
443 *Phytochemistry, 70*(11), 1345-1354.
444 Backenköhler, A., Eisenschmidt, D., Schneegans, N., Strieker, M., Brandt, W., & Wittstock, U.
445 (2018). Iron is a centrally bound cofactor of specifier proteins involved in glucosinolate
446 breakdown. *PLoS One, 13*(11), e0205755.
447 Baenas, N., Marhuenda, J., García-Viguera, C., Zafrilla, P., & Moreno, D. A. (2019). Influence
448 of cooking methods on glucosinolates and isothiocyanates content in novel cruciferous
449 foods. *Foods, 8*(7), 257.
450 Bernardi, R., Negri, A., Ronchi, S., & Palmieri, S. (2000). Isolation of the epithiospecifier protein
451 from oil-rape (*Brassica napus* ssp. *oleifera*) seed and its characterization. *FEBS*
452 *Letters, 467*(2–3), 296-298.
453 Statistisches Bundesamt (2018). https://www.destatis.de/ [access date 04/12/2019].
454 Chen, X., Hanschen, F. S., Neugart, S., Schreiner, M., Vargas, S. A., Gutschmann, B., &
455 Baldermann, S. (2019). Boiling and steaming induced changes in secondary
456 metabolites in three different cultivars of pak choi (*Brassica rapa* subsp. *chinensis*).
457 *Journal of Food Composition and Analysis, 82*, 103232.
458 Ciska, E., Drabińska, N., Honke, J., & Narwojsz, A. (2015). Boiled Brussels sprouts: A rich
459 source of glucosinolates and the corresponding nitriles. *Journal of Functional Foods,*
460 *19*, 91-99.
461 Ciska, E., & Kozłowska, H. (2001). The effect of cooking on the glucosinolates content in white
462 cabbage. *European Food Research and Technology, 212*(5), 582-587.
463 Ciska, E., Martyniak-Przybyszewska, B., & Kozłowska, H. (2000). Content of glucosinolates in
464 cruciferous vegetables grown at the same site for two years under different climatic
465 conditions. *Journal of Agricultural and Food Chemistry, 48*(7), 2862-2867.
466 Fechner, J., Kaufmann, M., Herz, C., Eisenschmidt, D., Lamy, E., Kroh, L. W., & Hanschen, F.
467 S. (2018). The major glucosinolate hydrolysis product in rocket (*Eruca sativa* L.),

468 sativin, is 1,3-thiazepane-2-thione: Elucidation of structure, bioactivity, and stability
469 compared to other rocket isothiocyanates. *Food Chemistry, 261*, 57-65.
470 Giambanelli, E., Verkerk, R., D'Antuono, L. F., & Oliviero, T. (2016). The kinetic of key
471 phytochemical compounds of non-heading and heading leafy *Brassica oleracea*
472 landraces as affected by traditional cooking methods. *Journal of the Science of Food*
473 *and Agriculture, 96*(14), 4772-4784.
474 Gronowitz, S., Svensson, L., & Ohlson, R. (1978). Studies of some nonenzymic reactions of
475 progoitrin. *Journal of Agricultural and Food Chemistry, 26*(4), 887-890.
476 Hanschen, F. S., Bauer, A., Mewis, I., Keil, C., Schreiner, M., Rohn, S., & Kroh, L. W. (2012).
477 Thermally induced degradation of aliphatic glucosinolates - Identification of
478 intermediary breakdown products and proposed degradation pathways. *Journal of*
479 *Agricultural and Food Chemistry, 60*(39), 9890-9899.
480 Hanschen, F. S., Herz, C., Schlotz, N., Kupke, F., Bartolomé Rodríguez, M. M., Schreiner, M.,
481 Rohn, S., & Lamy, E. (2015). The *Brassica* epithionitrile 1-cyano-2,3-epithiopropane
482 triggers cell death in human liver cancer cells in vitro. *Molecular Nutrition & Food*
483 *Research, 59*(11), 2178-2189.
484 Hanschen, F. S., Kaufmann, M., Kupke, F., Hackl, T., Kroh, L. W., Rohn, S., & Schreiner, M.
485 (2018). *Brassica* vegetables as sources of epithionitriles: Novel secondary products
486 formed during cooking. *Food Chemistry, 245*, 564-569.
487 Hanschen, F. S., Klopsch, R., Oliviero, T., Schreiner, M., Verkerk, R., & Dekker, M. (2017).
488 Optimizing isothiocyanate formation during enzymatic glucosinolate breakdown by
489 adjusting pH value, temperature and dilution in *Brassica* vegetables and *Arabidopsis*
490 *thaliana*. *Scientific Reports, 7*, 40807.
491 Hanschen, F. S., Kühn, C., Nickel, M., Rohn, S., & Dekker, M. (2018). Leaching and
492 degradation kinetics of glucosinolates during boiling of *Brassica oleracea* vegetables
493 and the formation of their breakdown products. *Food Chemistry, 263*, 240-250.
494 Hanschen, F. S., Platz, S., Mewis, I., Schreiner, M., Rohn, S., & Kroh, L. W. (2012). Thermally
495 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.

Hanschen, F. S., Rohn, S., Mewis, I., Schreiner, M., & Kroh, L. W. (2012). Influence of the chemical structure on the thermal degradation of the glucosinolates in broccoli sprouts. *Food Chemistry, 130*(1), 1-8.

Hanschen, F. S., & Schreiner, M. (2017). Isothiocyanates, nitriles, and epithionitriles from glucosinolates are affected by genotype and developmental stage in *Brassica oleracea* varieties. *Frontiers in Plant Science, 8*(1095), 1095.

Hopkins, R. J., Dam, M. N., & Van Loon, J. J. A. (2009). Role of glucosinolates in insect-plant relationships and multitrophic interactions. *Annual Review of Entomology, 54*.

Jones, R. B., Frisina, C. L., Winkler, S., Imsic, M., & Tomkins, R. B. (2010). Cooking method significantly effects glucosinolate content and sulforaphane production in broccoli florets. *Food Chemistry, 123*(2), 237-242.

Klopsch, R., Witzel, K., Börner, A., Schreiner, M., & Hanschen, F. S. (2017). Metabolic profiling of glucosinolates and their hydrolysis products in a germplasm collection of *Brassica rapa* turnips. *Food Research International, 100*(Pt 3), 392-403.

Kolonel, L. N., Hankin, J. H., Whittemore, A. S., Wu, A. H., Gallagher, R. P., Wilkens, L. R., John, E. M., Howe, G. R., Dreon, D. M., West, D. W., & Paffenbarger, R. S. (2000). Vegetables, fruits, legumes and prostate cancer: A multiethnic case-control study. *Cancer Epidemiology Biomarkers & Prevention, 9*(8), 795-804.

Kyung, K. H., Fleming, H. P., Young, C. T., & Haney, C. A. (1995). 1-Cyano-2,3-epithiopropane as the primary sinigrin hydrolysis product of fresh cabbage. *Journal of Food Science, 60*(1), 157-159.

Liou, C. S., Sirk, S. J., Diaz, C. A. C., Klein, A. P., Fischer, C. R., Higginbottom, S. K., Erez, A., Donia, M. S., Sonnenburg, J. L., & Sattely, E. S. (2020). A metabolic pathway for activation of dietary glucosinolates by a human gut symbiont. *Cell, 180*(4), 717-728.e719.

523 Matusheski, N. V., & Jeffery, E. H. (2001). Comparison of the bioactivity of two glucoraphanin
524 hydrolysis products found in broccoli, sulforaphane and sulforaphane nitrile. *Journal of*
525 *Agricultural and Food Chemistry, 49*(12), 5743-5749.
526 Matusheski, N. V., Juvik, J. A., & Jeffery, E. H. (2004). Heating decreases epithiospecifier
527 protein activity and increases sulforaphane formation in broccoli. *Phytochemistry,*
528 *65*(9), 1273-1281.
529 Matusheski, N. V., Swarup, R., Juvik, J. A., Mithen, R., Bennett, M., & Jeffery, E. H. (2006).
530 Epithiospecifier protein from broccoli (*Brassica oleracea* L. ssp. *italica*) inhibits
531 formation of the anticancer agent sulforaphane. *Journal of Agricultural and Food*
532 *Chemistry, 54*(6), 2069-2076.
533 Oerlemans, K., Barrett, D. M., Suades, C. B., Verkerk, R., & Dekker, M. (2006). Thermal
534 degradation of glucosinolates in red cabbage. *Food Chemistry, 95*(1), 19-29.
535 Palliyaguru, D. L., Yuan, J.-M., Kensler, T. W., & Fahey, J. W. (2018). Isothiocyanates:
536 Translating the power of plants to people. *Molecular Nutrition & Food Research, 62*(18),
537 1700965.
538 Sarvan, I., Verkerk, R., & Dekker, M. (2012). Modelling the fate of glucosinolates during thermal
539 processing of *Brassica* vegetables. *LWT - Food Science and Technology, 49*(2), 178-
540 183.
541 Springett, M. B., & Adams, J. B. (1989). Properties of Brussels sprouts thioglucosidase. *Food*
542 *Chemistry, 33*(3), 173-186.
543 Terry, P., Wolk, A., Persson, I., & Magnusson, C. (2001). *Brassica* vegetables and breast
544 cancer risk. *JAMA, 285*(23), 2975-2977.
545 Tookey, H. L. (1973). Crambe thioglucoside glucohydrolase (EC 3.2.3.1): separation of a
546 protein required for epithiobutane formation. *Canadian Journal of Biochemistry, 51*(12),
547 1654-1660.
548 Veeranki, O., Bhattacharya, A., Tang, L., Marshall, J., & Zhang, Y. (2015). Cruciferous
549 vegetables, isothiocyanates, and prevention of bladder cancer. *Current Pharmacology*
550 *Reports, 1*(4), 272-282.

551 Volden, J., Borge, G. I. A., Hansen, M., Wicklund, T., & Bengtsson, G. B. (2009). Processing
552 (blanching, boiling, steaming) effects on the content of glucosinolates and antioxidant-
553 related parameters in cauliflower (*Brassica oleracea* L. ssp. *botrytis*). *LWT - Food*
554 *Science and Technology, 42*(1), 63-73.
555 Wiesner, M., Zrenner, R., Krumbein, A., Glatt, H., & Schreiner, M. (2013). Genotypic variation
556 of the glucosinolate profile in pak choi (*Brassica rapa* ssp. *chinensis*). *Journal of*
557 *Agricultural and Food Chemistry, 61*(8), 1943-1953.
558 Witzel, K., Abu Risha, M., Albers, P., Börnke, F., & Hanschen, F. S. (2019). Identification and
559 characterization of three epithiospecifier protein isoforms in *Brassica oleracea*.
560 *Frontiers in Plant Science, 10*(1552).
561 Yen, G.-C., & Wei, Q.-K. (1993). Myrosinase activity and total glucosinolate content of
562 cruciferous vegetables, and some properties of cabbage myrosinase in Taiwan.
563 *Journal of the Science of Food and Agriculture, 61*(4), 471-475.

564

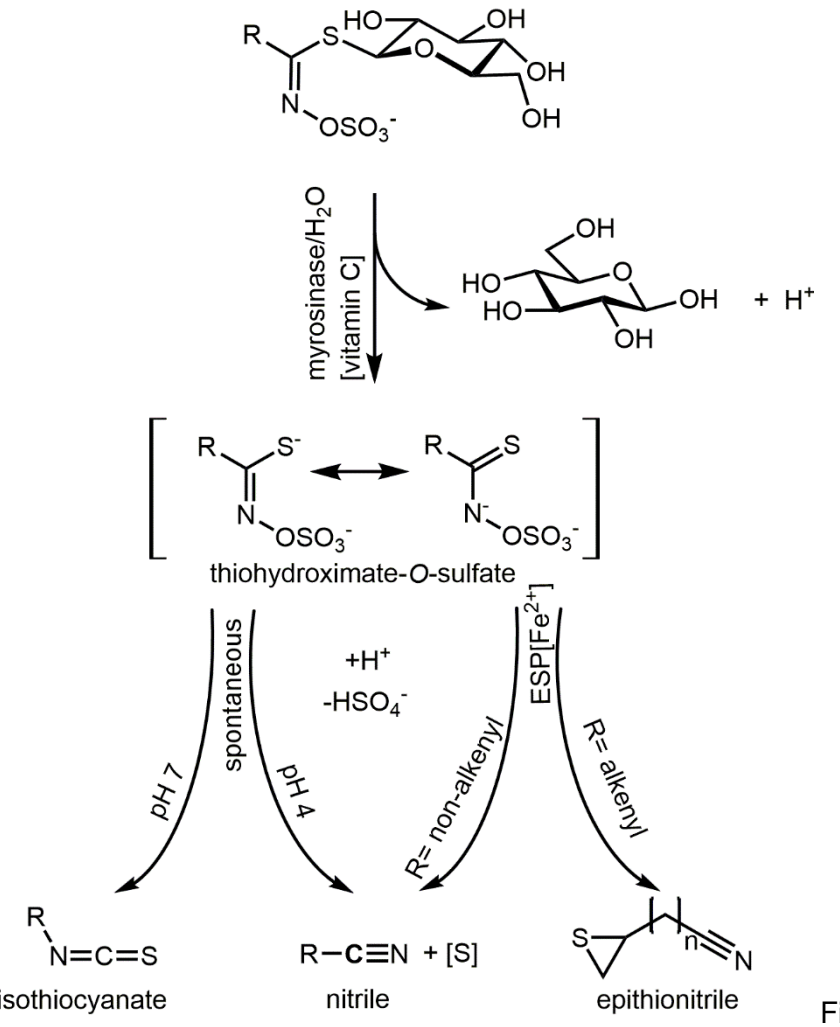
565

## Figure legends

Figure 1: The enzymatic hydrolysis of glucosinolates in *Brassica oleracea*. R-side chain, ESP: epithiospecifier protein

Figure 2: The effect of vinegar or lemon juice addition on glucosinolate (GLS) levels and their hydrolysis products in red cabbage salad (A) and of the ratio of isothiocyanate (ITC), nitrile (CN) and epithionitrile (ETN) levels relative to all hydrolysis products (B). Values represent mean ± standard deviation of four independent experiments (n=4). Significant differences in means between the formation of ITC, CN, or ETN (small letters, tested for each compound individually) as influenced by the different treatments with "H" being homogenized material and "NH" being non-homogenized salad were tested by the ANOVA and Tukey HSD test at the $p \leq 0.05$ level. Capital letters represent differences in total alk(en)yl GLS between control (water), vinegar, or lemon juice salad, as tested by the ANOVA and Tukey HSD test at the $p \leq 0.05$ level. FW = fresh weight.

Figure 3: Effect of red cabbage cooking (A) with vinegar or (B) with lemon juice and (C) blue cabbage cooking by adding $NaHCO_3$ on total alk(en)yl glucosinolate (GLS) levels and GLS degradation products. Values represent mean ± standard deviation of three independent experiments (n=3). Significant differences in means between isothiocyanate (ITC), nitrile (CN), or epithionitrile (ETN) levels (small letters, tested for each compound individually) as influenced by the boiling time were tested by the ANOVA and Tukey HSD test at the $p \leq 0.05$ level. Capital letters represent differences in alk(en)yl GLS between the different treatment times, as tested by the ANOVA and Tukey HSD test at the $p \leq 0.05$ level. FW = fresh weight.




592 Figure 1

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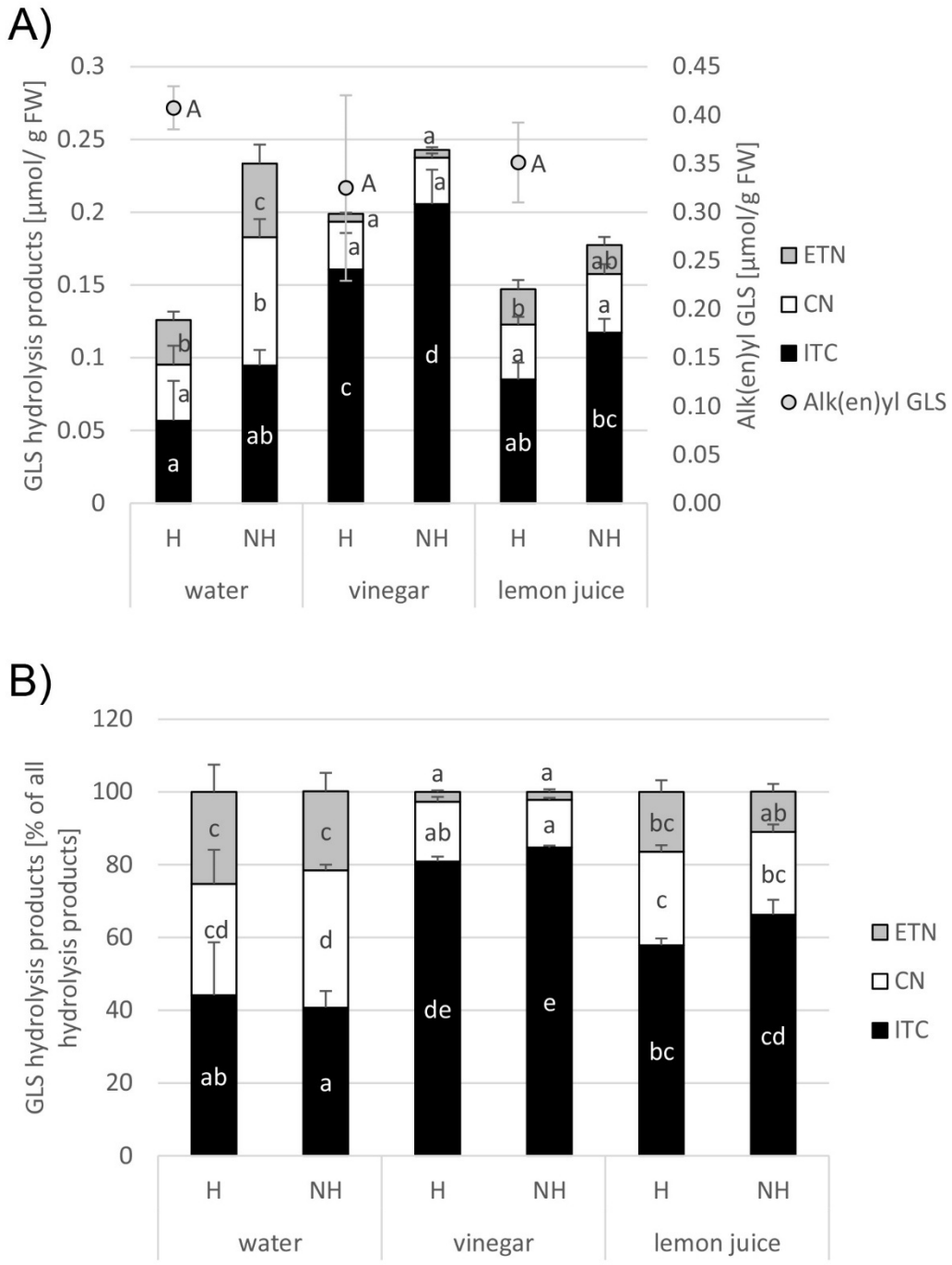
594




595 Figure 2

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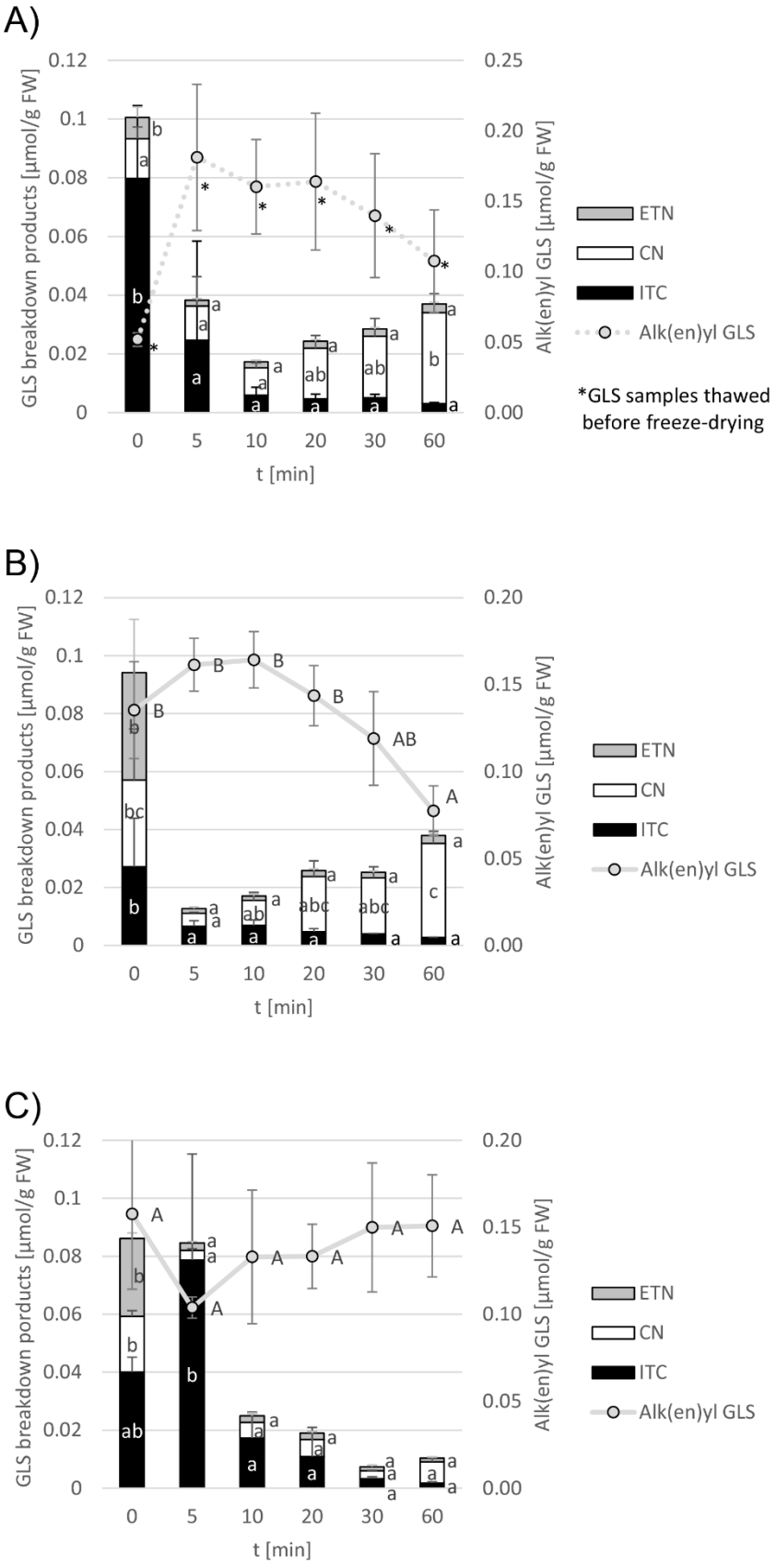

A)
GLS breakdown products [µmol/g FW]
Alk(en)yl GLS [µmol/g FW]
t [min]
ETN
CN
ITC
Alk(en)yl GLS
*GLS samples thawed before freeze-drying
B)
GLS breakdown products [µmol/g FW]
Alk(en)yl GLS [µmol/g FW]
t [min]
ETN
CN
ITC
Alk(en)yl GLS
C)
GLS breakdown porducts [µmol/g FW]
Alk(en)yl GLS [µmol/g FW]
t [min]
ETN
CN
ITC
Alk(en)yl GLS

Figure 3

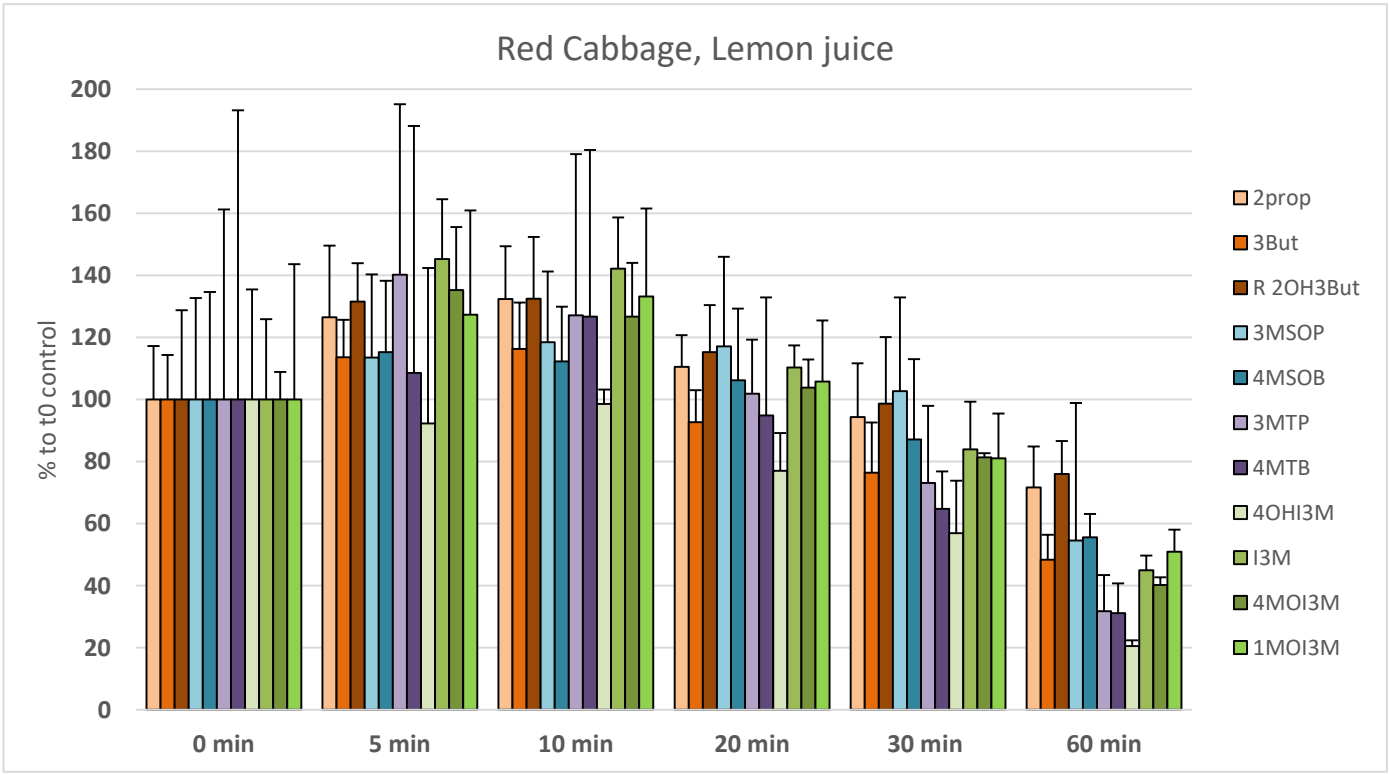
598 Table 1: PH values of red cabbage salad and boiled red cabbage prepared with vinegar, lemon juice or $NaHCO_3$ (boiling only).

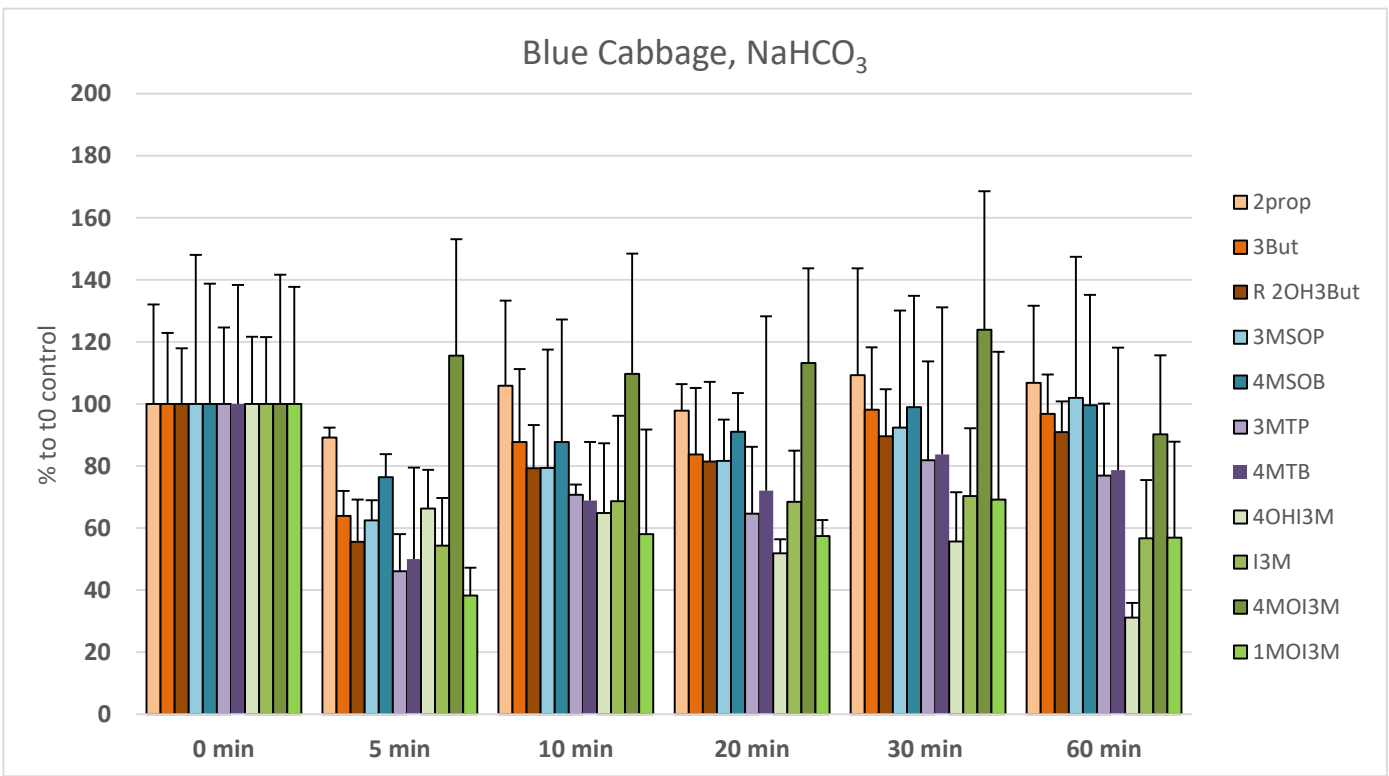
| | | Water | Vinegar | Lemon Juice | $NaHCO_3$ |
|---|---|---|---|---|---|
| Salad | Non-homogenized | 6.37 ± 0.13 a, C | 4.16 ± 0.17 a, A | 4.54 ± 0.10 a, B | – |
| | Homogenized | 6.38 ± 0.12 a, C | 4.29 ± 0.09 a, A | 4.75 ± 0.08 b, B | |
| Boiling | 0 min | | 4.23 ± 0.02 α, A | 4.30 ± 0.07 α, A | 6.82 ± 0.03 β, B |
| | 5 min | | 4.27 ± 0.02 αβγ, A | 4.43 ± 0.07 α, B | 6.83 ± 0.05 β, C |
| | 10 min | | 4.26 ± 0.03 αβ, A | 4.43 ± 0.06 α, B | 7.22 ± 0.08 γ, C |
| | 20 min | | 4.30 ± 0.02 βγ, A | 4.46 ± 0.04 α, B | 7.18 ± 0.04 γ, C |
| | 30 min | | 4.32 ± 0.02 γδ, A | 4.44 ± 0.05 α, A | 7.07 ± 0.08 γ, B |
| | 60 min | | 4.36 ± 0.02 δ, A | 4.43 ± 0.06 α, A | 6.56 ± 0.10 α, B |

599

600 Data presented are means ± standard deviation of three (boiling) or four (salad) independent experiments. Small letters indicate significant differences between
601 non-homogenized and homogenized salad as tested by t-test using STATISTICA software (version 13.2). Capital letters indicate significant differences in means
602 between the different additives (treatments); greek letters indicate significant differences between means of different boiling times as tested by one-way ANOVA
603 and Tukey HSD test using STATISTICA software (version 13.2).

604




Supplemental Figure 1: Glucosinolates (GLS) in boiled red cabbage after addition of lemon juice or $NaHCO_3$. 2Prop: allyl GLS, 3But: 3-butenyl GLS, R 2OH3But: (2*R*)-2-hydroxy-3-butenyl GLS, 3MSOP: 3-(methylsulfinyl)propyl GLS, 4MSOB: 4-(methylsulfinyl)butyl GLS, 3MTP: 3-(methylsulfanyl)propyl GSL, 4MTB: 4-(methylsulfanyl)butyl GLS, I3M: indol-3-ylmethyl GLS, 1MOI3M: 1-methoxyindol-3-ylmethyl GLS; 4MOI3M: 4-methoxyindol-3-ylmethyl GLS; 4OHI3M: 4-hydroxyindol-3-ylmethyl GLS