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Changes in Glucosinolate Concentrations, Myrosinase Activity, and Production of Metabolites of Glucosinolates in Cabbage (*Brassica oleracea* Var. *capitata*) Cooked for Different Durations

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In cabbage, glucosinolates such as sinigrin are hydrolyzed by plant myrosinase to allyl isothiocyanate (AITC), allyl cyanide, and, in the presence of an epithiospecifier protein, 1-cyano-2,3-epithiopropane (CEP). Isothiocyanates have been implicated in the cancer-protective effects of *Brassica* vegetables. The effect of processing on the hydrolysis of glucosinolates was investigated in cabbage. Cabbage was steamed or microwaved for six time durations over 7 min. Glucosinolate concentrations were slightly reduced after microwave cooking ($P < 0.001$) but were not influenced after steaming ($P < 0.05$). Myrosinase activity was effectively lost after 2 min of microwave cooking and after 7 min of steaming. Hydrolysis of residual glucosinolates following cooking yielded predominantly CEP at short cooking durations and AITC at longer durations until myrosinase activity was lost. Lightly cooked cabbage produced the highest yield of AITC on hydrolysis in vitro, suggesting that cooking *Brassica* vegetables for a relatively short duration may be desirable from a health perspective.

KEYWORDS: Steaming; microwaving; glucosinolates; myrosinase; cabbage; allyl isothiocyanate; 1-cyano-2,3-epithiopropane; epithiospecifier protein

INTRODUCTION

A large body of epidemiological evidence has indicated that the protective effects of *Brassica* vegetables against cancers of the alimentary tract and lungs may be partly due to their high content of glucosinolates (*1*). Glucosinolates are thioglucosides present in all families of *Brassica*. When the plant tissues are disrupted during processing, chewing, and digestion, glucosinolates become exposed to, and are hydrolyzed by, an endogenous thioglucosidase (EC 3.2.3.1), myrosinase, which is located within the vacuoles of the plant matrix. The hydrolysis generates an unstable aglycone intermediate, thiohydroxamate-*O*-sulfonate, which is spontaneously converted to different classes of breakdown products including isothiocyanates, thiocyanates, nitriles, epithionitriles, hydroxynitriles, and oxazolidine-2-thiones (*2*). One of the principal forms of chemoprotection, however, is thought to arise from isothiocyanates, which may influence the process of carcinogenesis partly by inhibiting phase I and inducing phase II xenobiotic metabolizing enzyme activity (*3*).

The extent of hydrolysis of glucosinolates and the nature and composition of the breakdown products formed are known to be influenced by various characteristics of the hydrolysis medium. Intrinsic factors such as coexisting myrosinase and its cofactors ascorbic acid, epithiospecifier protein (ESP), or ferrous ions (*4*) and extrinsic factors such as pH and temperature (*5*) can affect the hydrolysis of glucosinolates. In this respect, processing methods such as cutting and cooking are likely to influence the extent of glucosinolate hydrolysis and the ratio of the derivatives produced (*6*).

Earlier work has indicated that the glucosinolate−myrosinase system is modified during the processing of *Brassica* vegetables due to partial or total inactivation of myrosinase, thermal breakdown of glucosinolates and their hydrolysis products, loss of enzymic cofactors, leaching of glucosinolates and their derivatives into the cooking medium, or volatilization of the derivatives (*7*). The extent of these losses probably depends on the duration and type of heat treatment, the degree of material disintegration, and the vegetable matrix itself (*8*).

The study of the effects of processing on the concentrations of glucosinolates and the parameters related to their hydrolysis in *Brassica* vegetables has a pivotal role in complementing research on the epidemiology of the consumption of *Brassica* vegetables and chemoprevention. An understanding of the physical and biochemical changes occurring before the ingestion of processed *Brassica* vegetables may help to interpret the metabolic fate of glucosinolates in experimental studies in animals and humans and inform the subsequent formulation of dietary strategies to optimize the uptake of isothiocyanates in vivo.

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Only a few studies have provided a mechanistic understanding of the effects of cooking on the glucosinolate concentrations and/or myrosinase activity of *Brassica* vegetables (*9*−*11*). Furthermore, information regarding the effect of the extent of cooking on the glucosinolate-related characteristics of *Brassica*, and relating the residual glucosinolate concentrations and myrosinase activity to the formation of derivatives of glucosinolates produced, is scarce. The variations due to growing conditions and cultivar (*7*) as well as the different methods of food processing and analytical techniques used between studies make direct comparison and interpretation of the data difficult.

Processing methods such as microwaving or steaming are claimed to minimize nutrient losses while providing better physical quality of the food as compared with conventional cooking (*12*). The aim of the current experiment was to determine the effects of cooking treatment and time on the concentrations of residual glucosinolates and myrosinase activity in microwaved or steamed cabbage and on the potential yield of the derivatives of sinigrin from the in vitro hydrolysis of sinigrin in cooked cabbage. Sinigrin, the main alkenyl glucosinolate in cabbage, produces allyl isothiocyanate (AITC), allyl cyanide, and, in the presence of an epithiospecifier protein (ESP), 1-cyano-2,3-epithiopropane (CEP) when hydrolyzed by myrosinase. ESP is a small labile protein, which acts as a nonenzymic cofactor of myrosinase in the formation of cyanoepithioalkanes (*13*).

MATERIALS AND METHODS

Sampling and Processing of Cabbage. Six cabbages (*B. oleracea* var. *capitata* cv. Marathon), with a mean weight of 2566 (SEM 160.3) g, were sourced from a local supplier (Cocklaw Mains, Peterhead, Scotland) and stored at 4 °C until processing was complete. Each cabbage was cut into at least 12 evenly sized longitudinal wedges weighing around 120 g. Each of the 12 wedges from an individual cabbage was subjected to either microwave cooking or steaming for one time period of either 0, 45, 120, 210, 315, or 420 s. The longest time period was based on preliminary experiments determining the influence of different durations of cooking on the texture of cabbage and its suitability for consumption. Microwaving was conducted at 750 W (Whirlpool UK Ltd., Surrey, England) in a heat-resistant dish containing 16 mL of water and covered with pierced PVC cooking film. Steaming was undertaken in the bottom compartment of an electric pressureless steamer at 700 W (Russell Hobbs model 3501, Manchester, England). Boiling water was added to the water compartment of the steamer, and it was maintained at its boiling point throughout cooking. The duration of steaming was pre-set by a timer. The temperature of cabbage was recorded immediately after cooking ceased by inserting a food temperature probe at a depth of 50 mm into the center of the wedge of cooked cabbage from its outermost layer. The cooked samples were frozen by immersion in liquid nitrogen within 2 min after cooking and stored at −70 °C.

Analysis of Glucosinolate Concentrations by Reversed-Phase High-Performance Liquid Chromatography (HPLC). Around 80 g of each frozen cabbage sample was freeze-dried to constant dry weight. The extraction and analysis of desulfoglucosinolates were adapted from refs *14* and *15*. Freeze-dried cabbage (1 g), to which was added 200 μL of 12.5 mM benzyl glucosinolate in deionized water as an internal standard, was extracted twice with boiling 70% methanol in a water bath at 80 °C for 15 min. The suspension was centrifuged at 2500 rpm for 5 min at 22 °C after each extraction, and the supernatants were pooled. Methanol was removed from the pooled supernatant using a rotary evaporator, and the aqueous residue was made up to a final volume of 10 mL with deionized water.

Glucosinolates contained in the extracts were enzymatically desulfated overnight by 0.2% aryl sulfatase (Sigma, St. Louis, MO) on columns of DEAE-Sephadex A-25 (Sigma, St. Louis, MO), pre-washed with 0.5 and 0.02 M pyridine acetate buffer (*15*). Desulfoglucosinolates were eluted from the column with 2 mL of deionized water and separated on an Agilent 1100 Series HPLC system (Agilent Technologies, Waldbronn, Germany) with a LiChrosphere RP-18 endcapped column, 250 mm × 4 mm and particle size 5 μm (Merck, Darmstadt, Germany). The mobile phase consisted of a mixture of water and acetonitrile at a flow rate of 1 mL/min, starting with 100% water for 1 min to reach 20% acetonitrile at 46 min for 8 min and back to 100% water at 58 min for 7 min. Individual desulfoglucosinolates were detected using a diode array detector at 228 nm and were identified by their respective retention times and the characteristics of their UV spectra from 190 to 400 nm.

ChemStation Rev. A.10.02 (Agilent Technologies, Waldbronn, Germany) was used to identify and quantify glucosinolates. Identification was confirmed by a combination of authentic standards and the analysis of a selection of *Brassica* vegetables with reference to the literature. Individual glucosinolates were quantified using published response factors that have been experimentally determined (*14*). A standard curve was constructed for sinigrin (Sigma- Aldrich, St. Louis, MO) at concentrations from 0 to 20 μmol/nominal g of DM (dry matter) within a freeze-dried watercress matrix ($r^2 = 0.9982$). The limit of quantification for desulfoglucosinolates was 0.044 μmol. The coefficient of variation of extraction and analysis of desulfoglucosinolates from cabbage ($n = 6$) was 4.5% for sinigrin and 2.5% for total glucosinolates.

Determination of Myrosinase Activity. Myrosinase activity in cabbage was determined by measuring the rate of disappearance of sinigrin using a UV−vis spectrophotometer (Cary 50, Varian Ltd., Surrey, England) according to ref *16*, with the following modifications. The reaction mixture contained 150 μM sinigrin (Sigma-Aldrich, St. Louis, MO), and its degradation was measured at 227 nm. A standard curve was prepared using myrosinase from a commercial source (Sigma-Aldrich, St. Louis, MO) and found to be linear over the range of myrosinase concentrations present in the cabbage samples ($r^2 = 0.9822-0.9924$). The coefficient of variation for a series of replicates ($n = 7$) was 4%.

Analysis of the Formation of Derivatives of Sinigrin by Gas Chromatography Following Its Hydrolysis in Cooked Cabbage in Vitro. Sinigrin in cooked cabbage was hydrolyzed by plant myrosinase by incubating 0.1 g of freeze-dried cabbage in 2 mL of deionized water, containing 100 μL of 5 mM benzyl isothiocyanate (Aldrich Chemical Co., Milwaukee, WI) made up in ethanol as an internal standard, for 5 min at 37 °C. The hydrolysate was extracted twice with 5 mL of dichloromethane and centrifuged at 2500 rpm for 5 min. The dichloromethane extracts were pooled and concentrated to 200 μL under a stream of air. The extracts were analyzed on a Hewlett-Packard 5890 GC and detected by a flame ionization detector. The column was an Equity-1 fused silica capillary column with a nonpolar poly(dimethylsiloxane) bonded phase, 30 m × 0.25 mm i.d. × 0.25 μm film thickness (Supelco, Bellefonte, PA). The initial temperature of the column was maintained at 35 °C for 3 min and then increased linearly at 6 °C/min to 160 °C followed by a second ramp of 10 °C/min to reach a temperature of 200 °C. This final temperature was maintained for 4 min. Helium was used as the carrier gas at a constant flow rate of 1 mL/min. The injector and detector were held at 200 °C. The injector was set in split/splitless mode, with a splitless time of 30 s and a split flow of 30 mL/min.

ChemStation Rev. A.10.02 (Agilent Technologies, Waldbronn, Germany) was used to identify and quantify the derivatives. Authentic standards of AITC and allyl cyanide (Sigma-Aldrich, St. Louis, MO) were extracted in dichloromethane, within a freeze-dried watercress matrix, at nominal concentrations from 0 to 0.0094 μmol/100 mg of vegetable, using 100 μL of 5 mM benzyl isothiocyanate as the internal standard. Response factors for allyl cyanide and AITC relative to benzyl isothiocyanate were 1.47 and 0.75, respectively, and r^2 values for their calibration curves were 0.9987 and 0.9999, respectively. Because of the lack of a commercial standard for CEP, we identified this derivative by the analysis of dichloromethane extracts from hydrolyzed raw cabbage using a Finnigan Trace DSQ single quadrupole GC-MS system (Thermo Electron Corporation, Hertfordshire, UK). We used similar GC conditions as stated previously and compared the mass spectrum of 1-cyano-2,3-epithiopropane with published data (*17*). The mass spectrum data for the putative CEP peak had *m*/*z* (%) values: 39 (5.4), 44.9 (11), 54 (16.23), 58.9 (35.6), 71.9 (39.8), and 98.9 (M^+, 100),

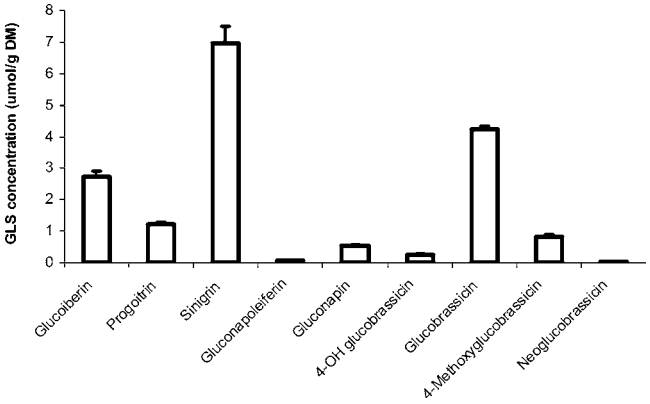

Figure 1. Profile of glucosinolates (GLS) in raw cabbage cv. Marathon. Vertical bars represent standard error of the mean.

thus corresponding to the published spectrum (*17*). We quantified 1-cyano-2,3-epithiopropane using the response factor for the simple nitrile derivative, allyl cyanide, assuming them to have a similar response in the FID. Coefficients of variation for the analysis of allyl cyanide, AITC, and CEP were 2.9, 4.4, and 4.0%, respectively, for a series of replicate samples ($n = 6$).

Statistical Analysis. The effects of cooking treatment, cooking time, and their interaction on the concentrations of glucosinolates, residual myrosinase activity, and the formation of the derivatives of sinigrin on hydrolysis of cooked cabbage were tested by two-way ANOVA. Since each cabbage was divided into 12 portions that were each subjected to one of the different combinations of cooking treatment and time (two cooking treatments for six durations), we were able to treat individual cabbage heads as blocks in the analysis of variance and thus to account for background variation due to cabbage heads. The order of cooking method and time within each block was randomized so that each cooking method and cooking time appeared as an equal number of times in the rows and columns of the treatment matrix. This allowed us to account for any variation due to the order of processing samples. Results were expressed as means and standard errors of the mean (SEM) of six samples analyzed in duplicate. The difference between the means of certain cooking times on myrosinase activity and formation of the derivatives of sinigrin was tested using orthogonal contrasts within ANOVA at $P < 0.001$. All statistical analyses were performed using the statistical package GenStat Release 8.1 (*18*).

RESULTS

Glucosinolate Concentrations. The range of glucosinolate concentrations, expressed as μmol/g of DM, in raw cabbage (cv. Marathon) is shown in **Figure 1**. Sinigrin accounted for 41.3% of the total glucosinolates, with a mean concentration of 6.98 (SEM 0.518) μmol/g of DM. **Figure 2** illustrates the concentration of sinigrin, expressed as a proportion of total glucosinolates, in cooked cabbage at the different stages of processing. Total glucosinolate concentrations were significantly affected by cooking time ($P < 0.001$) and cooking treatment ($P < 0.05$). Orthogonal contrast analysis of the mean concentrations showed that the concentration of total glucosinolates was significantly reduced by 12.4% ($P < 0.05$) and 17.3% ($P < 0.001$) after microwaving cabbage for 315 and 420 s, respectively. The glucosinolates responsible for the significant reduction in total glucosinolates during microwaving over 7 min (420 s) were the alkenyl glucosinolates sinigrin (reduction of 22.3%, $P < 0.05$) and gluconapin (reduction of 18.5%, $P < 0.05$) and the indole glucosinolates 4-hydroxyglucobrassicin (reduction of 22.7%, $P < 0.05$) and glucobrassicin (reduction of 12.3%, $P < 0.05$). The alkenyl glucosinolates glucoiberin and progoitrin remained unchanged. There was no significant change in the concentration of sinigrin or total glucosinolates during steaming.

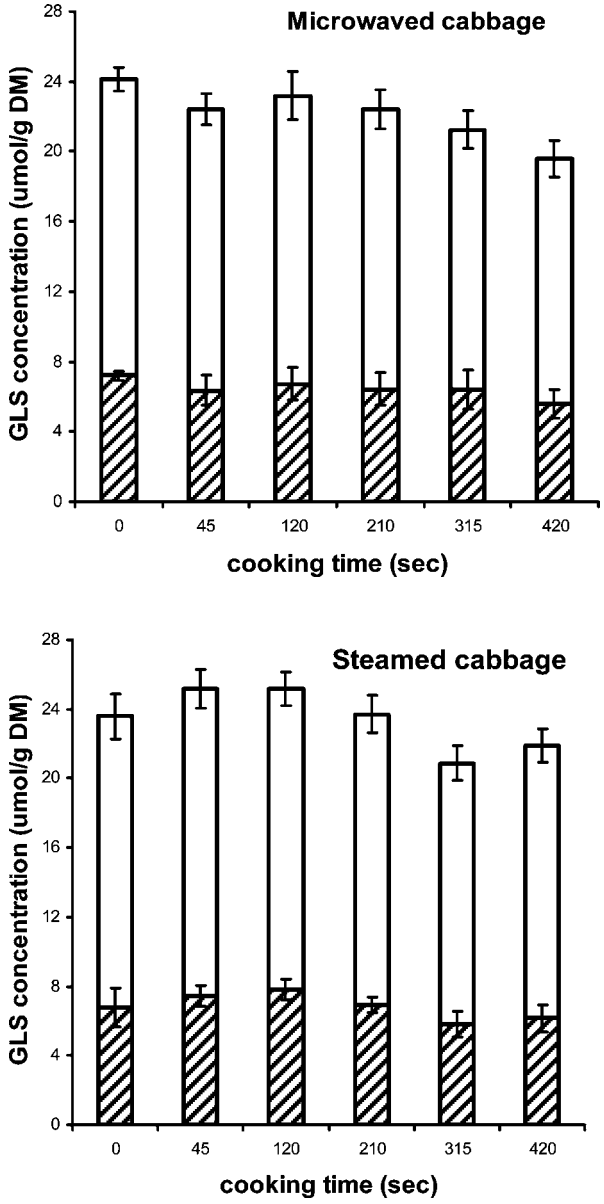

Figure 2. Mean concentration of sinigrin (umol/g DM) (shaded area) as a proportion of the total concentration of glucosinolates (□) in cooked cabbage at the six stages of processing. Vertical bars represent standard error of the mean. Total glucosinolate concentrations were significantly affected by cooking time ($P < 0.001$) and cooking treatment ($P < 0.05$).

Temperature. The mean internal temperature of microwaved cabbage increased from 6.5 °C (SEM 0.43 °C) in its raw state to 88.0 °C (SEM 4.20 °C) in samples microwaved for 120 s and reached a maximum temperature of 100.0 °C (SEM 0.45 °C) in cabbage cooked further. Steaming produced a gradual rise in mean internal temperature from 7.5 °C (SEM 0.76 °C) in raw cabbage to 68.22 °C (SEM 4.28 °C) in cabbage steamed for 420 s.

Myrosinase Activity. The activity of myrosinase in cooked cabbage was significantly influenced by cooking treatment ($P < 0.001$), cooking time ($P < 0.001$), and an interaction between the two factors ($P < 0.001$). Myrosinase in microwaved cabbage showed an initial significant decrease of 27.4% in activity after being cooked for 45 s and an abrupt reduction of 96.7% after cooking for 120 s, as compared with raw cabbage ($P < 0.001$). The activity then remained stable in cabbage cooked for subsequent times for up to 420 s (**Figure 3**).

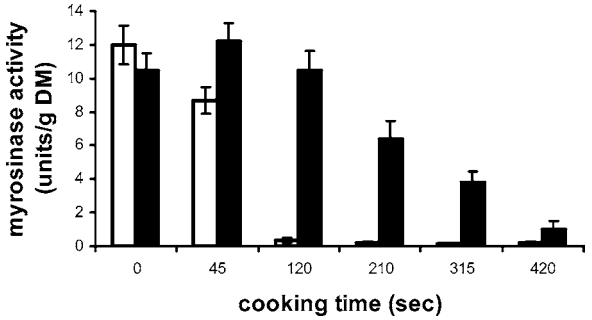

Figure 3. Mean myrosinase activity, expressed as units/g of dry matter (DM), in microwaved (□) or steamed (■) cabbage at each stage of processing. Vertical bars represent standard error of the mean. Myrosinase activity was significantly affected by cooking time ($P < 0.001$), cooking treatment ($P < 0.001$), and an interaction between cooking time and treatment ($P < 0.001$).

Conversely, the myrosinase activity in steamed cabbage remained unaltered after cooking for up to 120 s, as determined from orthogonal contrast analysis of their means in ANOVA, and thereafter showed a significant, gradual, and steady reduction for the remaining cooking times ($P < 0.001$). Raw cabbage showed a loss of 90.4% in myrosinase activity after being steamed for 420 s (**Figure 3**).

Derivatives of Sinigrin. When cabbage was hydrolyzed in water, the yield of the three breakdown products of sinigrin (AITC, allyl cyanide, and CEP) was significantly influenced by cooking method ($P < 0.001$), cooking time ($P < 0.001$), and an interaction between these factors ($P < 0.001$). The production of allyl cyanide from the hydrolysis of cabbage declined with cooking, with a marked reduction in its formation from cabbage microwaved for 120 s or steamed for 315 s onward (**Figure 4**).

The formation of CEP was maximal from raw cabbage (mean 0.194 (SEM 0.009) μmol/g of DM) and was reduced by 87% in cabbage microwaved for 120 s. It remained stable in cabbage microwaved for longer time periods (**Figure 4**). Conversely, the yield of AITC was minimal (mean 0.037 (SEM 0.007) μmol/g of DM) following the hydrolysis of raw cabbage. The production of AITC then increased in samples microwaved further until it reached its maximum production (mean 0.334 (SEM 0.041) mol/g of DM) in cabbage microwaved for 120 s. At this stage, the production of AITC from the hydrolysis of microwaved cabbage was increased by 88%, as compared with raw cabbage. The increase was compatible with the reduction (87%) in the concentration of CEP formed from cabbage microwaved for a similar time (**Figure 4**).

The production of CEP and allyl isothiocyanate from cabbage steamed for different times followed a similar pattern but at a slower rate to that of the microwaving treatment (**Figure 4**). The yield of CEP from cabbage steamed for up to 120 s remained steady (mean 0.181 (SEM 0.019) μmol/g of DM), followed by a gradual reduction in its formation from cabbage steamed for 210−420 s to attain a mean yield of 0.05 (SEM 0.016) μmol/g of DM). At this stage, the production of CEP was reduced by 76 %, as compared with raw cabbage. Similarly, the production of AITC from cabbage steamed at the different stages until 120 s (mean 0.043 (SEM 0.008) μmol/g of DM) was unaltered and, in contrast, was gradually increased from cabbage cooked for 210−420 s, where it reached a maximal mean yield of 0.303 (SEM 0.042) μmol/g of DM. The yield of AITC from cabbage steamed for 420 s min was increased by 578 %, as compared with raw cabbage.

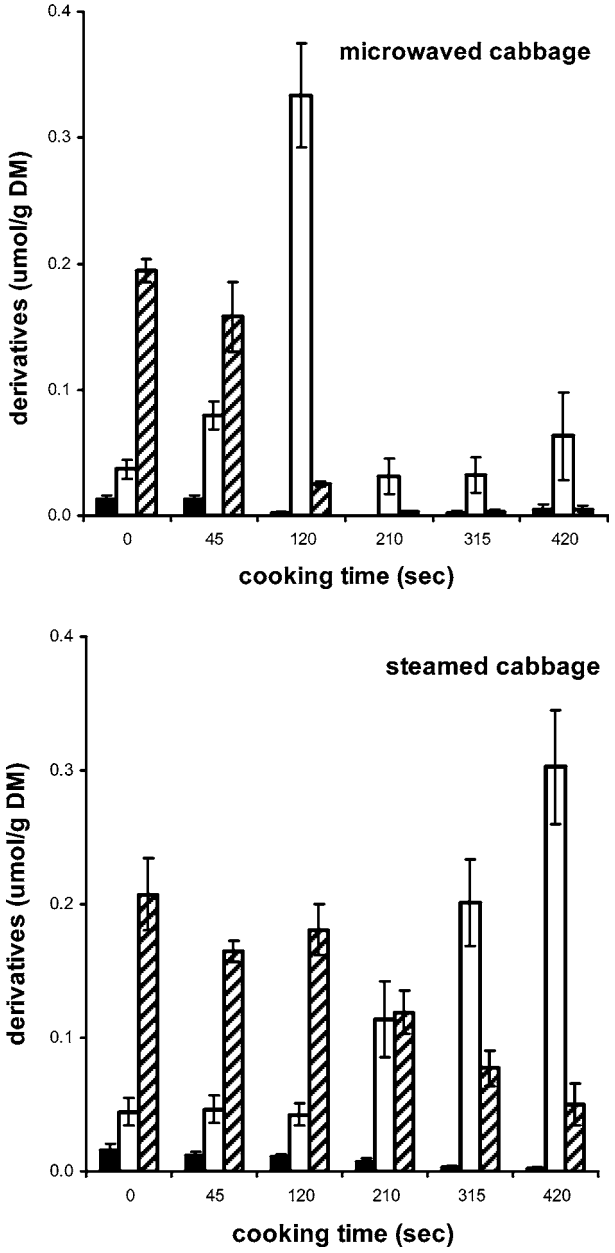

Figure 4. Mean concentration of allyl cyanide (■), allyl isothiocyanate (AITC) (□), and 1-cyano-2,3-epithiopropane (CEP) (shaded area), expressed as umol/g of dry matter (DM), on in vitro hydrolysis of cooked cabbage. Vertical bars represent standard error of the mean. Concentrations of derivatives were significantly affected by cooking time ($P < 0.001$), cooking treatment ($P < 0.001$), and an interaction between cooking time and treatment ($P < 0.001$).

DISCUSSION

The present study investigated the time-course effects of processing cabbage by steaming or microwaving on the concentrations of glucosinolates and residual myrosinase activity at six time intervals over 7 min. We also simulated the hydrolysis of sinigrin in vitro from cabbage cooked at these time points and determined the proportion of breakdown products formed.

The findings showed that the concentration of sinigrin and total glucosinolates was significantly reduced by 22.3 and 17.3%, respectively, at the end stages of the microwaving treatment but remained stable at the different stages of steaming. These results are not in agreement with previous reports. Vallejo

et al. (*9*) showed a reduction of 74% in the concentration of total glucosinolates when broccoli florets were microwaved for 5 min at 1000 W and explained this loss by a high degree of water loss that contained leached glucosinolates. However, the extent of water evaporation was not reported. In our experiment, cooked cabbage showed an average loss of 32% of its raw weight after being microwaved for 7 min. The lower loss in total glucosinolates observed in the current study could be partly due to a smaller surface area provided for heat penetration into a whole portion of cabbage wedge as compared with broccoli florets. We also used a lower output power in our microwaving treatment. Total glucosinolate concentrations following cooking have been shown to be influenced by energy input, which is a function of cooking time and output power, during microwaving (*11*). The difference in the profile of glucosinolates between and within *Brassica* species (*19*) may further contribute to these variations since variable stabilities of individual glucosinolates have been reported during the cooking of *Brassica* (*9*, *10*, *20*). Conversely, Verkerk and Dekker (*11*) demonstrated an increase of 78% in total glucosinolates after microwaving red cabbage for 4 min 48 s at 900 W. The authors speculated that the rise may be due to a higher chemical extractability of glucosinolates from cooked cabbage as compared with its raw counterpart, although this seems unlikely since glucosinolates are readily extracted into 70% methanol (Quinsac, unpublished). Vallejo et al. (*9*) showed a minimal reduction of 2% in the concentrations of total glucosinolates after steaming broccoli for 3.5 min, which is comparable to our findings in cabbage.

In the present study, the main contribution in the reduction in total glucosinolate concentrations after microwaving cabbage for 7 min was from the alkenyl glucosinolates sinigrin and gluconapin and the indole glucosinolates 4-hydroxyglucobrassicin and glucobassicin. In contrast to the higher thermolability or increased rate of diffusion into the cooking medium of indole glucosinolates as compared with aliphatic glucosinolates reported in cooked *Brassica* (*9*, *10*, *21*), such characteristics were not observed in the present study. Among the glucosinolates that underwent a significant decrease in concentrations after microwaving cabbage for 7 min, glucobrassicin, the predominant indole glucosinolate in cabbage, was the least thermolabile. Sinigrin, gluconapin, and 4-hydroxyglucobrassicin were significantly reduced by similar amounts. High losses of glucoiberin and progoitrin have been reported in boiled cabbage due to their high thermolability or increased rate of diffusion into the cooking medium (*8*, *10*). These aliphatic glucosinolates were unaffected by cooking in the current study. The discrepancies in the nature and magnitude of changes in glucosinolate concentrations observed in cooked *Brassica* vegetables between these studies and the present experiment may partly, although not largely, be accounted for by variations attributed to vegetable matrix, cultivar, agronomic conditions (*22*), or subtle differences in sampling, cooking protocols, or analytical techniques. During cooking, changes in glucosinolate concentrations are also related to the residual activity of myrosinase. Both glucosinolate concentrations and myrosinase activity have been shown to vary extensively between different cultivars of the same species of *Brassica* vegetables (*23*).

The activity of myrosinase in cabbage was markedly influenced by cooking treatment and time. Myrosinase activity in cabbage at the different stages of cooking was negatively associated with cooking time and the corresponding internal temperature of the cooked product. Microwave cooking can generate rapid temperature rises within the food matrix (*24*). A two-step model has been proposed for the inactivation of myrosinase, consisting of a fast initial inactivation period followed by a slower decay, which finally levels out (*25*). This was demonstrated by the abrupt reduction in myrosinase activity after microwaving cabbage for 120 s, with a corresponding marked increase to 91 °C in the temperature of the cooked cabbage. The myrosinase activity in cabbage microwaved for longer remained minimal and stable, once the temperature had reached 100 °C. These observations are similar to those of Verkerk and Dekker (*11*) when red cabbage was microwaved at 900 W for up to 4 min 48 s. The authors demonstrated a sudden drop in myrosinase activity in microwaved cabbage at 144 s. In our study, the slower rate of thermal energy penetrating the core of cabbage during steaming produced a gradual and steady decrease in myrosinase activity in cooked cabbage at the different stages of steaming. Cabbage steamed for 420 s reached an average temperature of 68 °C after cooking and had a residual myrosinase activity 4.6-fold higher than the corresponding microwaved sample. The speed and extent of myrosinase inactivation over time between the two cooking treatments are related to the rate and principle of heat transfer. In conventional cooking such as steaming, heating starts at the surface of the food, and heat is slowly transferred to the center by conduction. Conversely, in microwave cooking, microwaves permeate the center of the food by radiation, and the heat generated within the food is transferred toward the surface of the food. In this respect, an equivalent rise in temperature occurs more quickly in microwave processing than steaming (*12*).

Sinigrin, in raw or cooked cabbage, was broken down in vitro in water at 37 °C to simulate the temperature within the gastrointestinal tract. Water was used as the medium for hydrolysis, instead of a buffered medium, since pH may vary widely along the gastrointestinal tract (*26*), and it is unclear where the majority of hydrolysis of glucosinolates occurs within the alimentary tract. A highly significant effect was observed on the relative concentration and ratio of the metabolites of sinigrin formed from its hydrolysis in cabbage cooked to different degrees. The reduction in myrosinase activity relative to the possible changes in ESP, and the physical and chemical changes within the *Brassica* matrix during steaming or at the first four durations of microwaving cabbage, may explain the different profile of metabolites produced. Allyl cyanide comprised the smallest proportion of derivatives produced on hydrolysis of cabbage. The main breakdown product formed from the hydrolysis of sinigrin in raw or near-raw cabbage was the nitrile derivative, CEP, which conforms to the observations from hydrolysis of raw cabbage in previous studies (*27*, *28*).

Cyanoepithioalkanes have been shown to be preferentially produced from the myrosinase-mediated hydrolysis of their precursor alkenyl glucosinolates such as sinigrin, instead of the corresponding isothiocyanates, in the presence of ESP (*29*). ESP may block the rearrangement of thiohydroxamate-*O*-sulfonate to isothiocyanates (*17*) or assist in the transfer of sulfur from the glucosinolate molecule to the terminal alkene residue of the side chain (*30*). Recently, Lambrix et al. (*30*) have reported that ESP may also promote the formation of simple nitriles from non-alkenyl glucosinolates. ESP activity was not measured in the current study due to unavailable commercial standards and no known means of directly measuring its activity. Recently, Matusheski et al. (*31*) studied the effect of heating on ESP activity in broccoli by measuring the relative amounts of cyanoepithioalkane and simple nitrile generated by the hydrolysis of purified epiprogoitrin with purified myrosinase, in the presence of the broccoli extract under investigation. They reported that ESP activity was significantly reduced at a

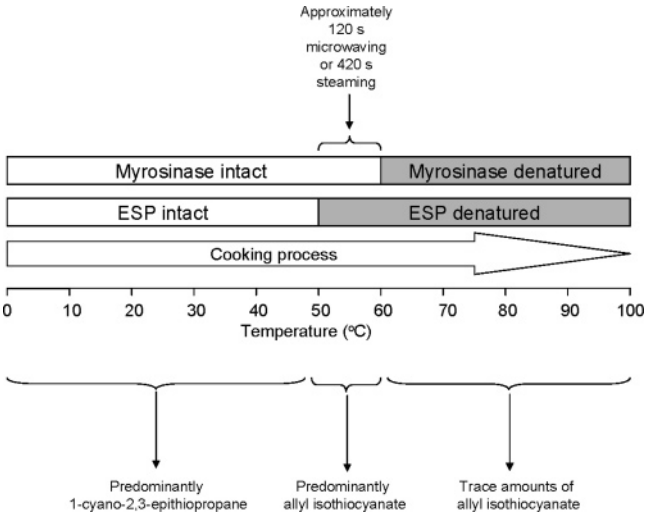

Figure 5. Schematic illustrating the proposed processes occurring during cooking of cabbage, which leads to the production of different predominant breakdown products at different stages of cooking. Temperatures should be treated as approximate.

temperature of 50 °C and above, with a corresponding reduction in the formation of the simple nitrile produced from the non-alkenyl glucosinolate, glucoraphnin, in broccoli. Our results are consistent with these findings. In our study, the marked reduction in the yield of CEP from cabbage microwaved for 120 s or steamed for 420 s corresponded to an internal temperature above 50 °C in cooked cabbage (**Figure 5**).

In contrast, the yield of AITC was minimal from raw and near-raw cabbage (microwaved up to 45 s or steamed up to 210 s), despite possessing the highest myrosinase activity. This may be due to the preferential formation of CEP from sinigrin in cabbage in the presence of residual ESP activity at these stages. Furthermore, it has been suggested that ESP may cause allosteric inhibition of myrosinase (*32*). With further cooking and denaturation of ESP, the production of AITC from the hydrolysis of cooked cabbage was increased proportionally to the reduction in formation of the cyanoepithioalkane. It is plausible that cabbage microwaved for 120 s or steamed for 420 s produced the highest yield of AITC on hydrolysis due to the denaturation of ESP at these cooking times despite the low myrosinase activity in these samples (**Figure 5**).

After the marked increase in AITC from the hydrolysis of sinigrin at early cooking times, a much smaller yield of isothiocyanates was produced in cabbage microwaved from 210 s onward, despite virtually no residual myrosinase activity in these samples. The formation of the derivatives of sinigrin produced by the in vitro hydrolysis of cabbage cooked at the different stages is essentially a function of residual concentrations of sinigrin and myrosinase activity in cooked cabbage during the hydrolysis phase. We anticipated that any breakdown products formed during cooking would be lost by volatilization. However, the presence of small concentrations of AITC from the hydrolysis of cabbage with no myrosinase activity suggests that the derivatives may not be so volatile when present within a rigid cellular structure as in lightly cooked cabbage but are more prone to volatilization upon further disruption of the tissues with prolonged cooking. In this respect, the highest yield of AITC from lightly cooked cabbage (microwaved for 120 s or steamed for 420 s) is more likely a combination of that produced during both the cooking and the in vitro hydrolysis phases. It is possible that AITC formed during cooking was bound to cabbage cell membranes and released from cell lysis during sample processing such as freeze-drying, grinding, and hydrolysis. Isothiocyanates have been shown to form conjugates with proteins (*33*).

Some of the cooking durations for both processing treatments did not reflect domestic practice. They had been chosen to determine and compare the time-course effects on the glucosinolate-related characteristics of cabbage after microwaving or steaming by using a series of cooking durations including those that would be used in domestic practice. Furthermore, the steaming treatment was different to conventional steaming of chopped cabbage over a pan of boiling water. We used an electric steamer and a wedge of cabbage to enable standardized cooking conditions similar to the microwaving treatment in terms of a predetermined cooking time and standardized cooking-energy efficiencies for uniformity of cooking.

Our findings suggest that during processing, the glucosinolate concentrations and residual myrosinase activity in cabbage are dependent on the method and duration of processing. Although sinigrin concentrations remained stable throughout steaming or for most of the microwaving treatment, the highest yield of AITC seems to be from lightly cooked cabbage with a low level of myrosinase activity, as compared with raw or overcooked cabbage. The consumption of lightly cooked *Brassica* vegetables may provide a means of optimizing the uptake of isothiocyanates in vivo. However, further studies investigating in vivo hydrolysis should be conducted to test if this observation is reproducible in the human digestive tract.

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