Glories, Y. Relations protéines-tanins. Le problème de l'astringence des vins rouges. *Proceedings, International Phenolic Group Convention*; INRA: Paris, 1982; Vol. 11, p 71.
Glories, Y. Mesure et définition de la couleur du vin. *Proceedings, International Phenolic Group Convention*; INRA: Paris, 1986; Vol. 13, p 394.
Jerumanis, J. Quantitative analysis of flavanoids in barley, hops and beer by high-performance liquid chromatography (HPLC). *J. Inst. Brew.* **1985**, *91*, 250.
Koh, K. H. Recherches sur l'activité estérase exocellulaire de *Botrytis cinerea* et sur l'action fongicide de l'acide hexanoïque. Sc. Doct. Thesis, University of Bordeaux, France, 1985.
Lea, A. G. H. The phenolics of ciders. Oligomeric and polymeric procyanidins. *J. Sci. Food Agric.* **1978**, *29*, 471.
Lea, A. G. H.; Bridle, P.; Timberlake, C. F.; Singleton, V. L. The Procyanidins of white grapes and wines. *Am. J. Enol. Vitic.* **1979**, *30*, 289.
Lee, C. Y.; Jaworski, A. Phenolic compounds in white grapes grown in New York. *Am. J. Enol. Vitic.* **1987**, *38*, 277.
Michaud, J.; Lagaze, P.; Masquelier, J. Fractionnement des Oligomères flavanoliques du raisin. *Bull. Soc. Pharm. Bordeaux* **1971**, *110*, 111.
Milhé, J. C. Recherches technologiques sur les composés phénoliques des vins rouges. Sc. Doct. Thesis, University of Bordeaux, France, 1969.
Mulkay, P.; Touillaux, R.; Jerumanis, J. Les prodelphinidines de l'orge. Séparation, identification et influence sur la stabilité colloïdale de la bière. *Cerevisia* **1981**, *1*, 29.
Oszmianski, J. Enzymatic changes in phenolic compounds in the model systems and fruit extracts. Sc. Doct. Thesis, University of Wroclaw, Poland, 1988.
Oszmianski, J.; Sapis, J. C.; Macheix, J. J. Changes in grape seed phenols as affected by enzymic and chemical oxidation in vitro. *J. Food Sci.* **1985**, *50*, 1505.
Oszmianski, J.; Romeyer, F. M.; Sapis, J. C.; Macheix, J. J. Grape seed phenolics. Extraction as affected by some conditions occurring during wine processing. *Am. J. Enol. Vitic.* **1986**, *37*, 7.
Outtrup, H. Structure of prodelphinidins in barley. *Eur. Brew. Conv. Proc.* **1981**, 332.
Piretti, M. V.; Ghedini, M.; Serazanetti, G. Isolation and identification of the polyphenolic and terpenoid constituents of *Vitis vinifera* Trebbiano. *Ann. Chim.* **1976**, *66*, 429.
Ribéreau-Gayon, P.; Milhé, J. C. Les Flavanes du raisin. *C.R. Seances Acad. Sci.* **1969**, *268*, 1813.
Romeyer, F. M. Les composés phénoliques du raisin *Vitis vinifera*. Evolution au cours de la maturation du fruit et conséquences technologiques. Sc. Doct. Thesis, University of Montpellier, France, 1984.
Romeyer, F. M.; Macheix, J. J.; Sapis, J. C. Grape seed phenolics. Changes and importance of oligomeric procyanidins during grape maturation. *Phytochemistry* **1986**, *25*, 219.
Rossi, J. A.; Singleton, V. L. Flavor effects and adsorptive properties of purified fractions of grape seed phenols. *Am. J. Enol. Vitic.* **1966**, *17*, 240.
Salagoïty-Auguste, M. H.; Bertrand, A. Wine phenolics. Analysis of low molecular weight components by high performance liquid chromatography. *J. Sci. Food Agric.* **1984**, *35*, 1241.
Singleton, V. L. Grape and wine phenolics. Background and prospects. *Proceedings, Centenial Davis University*, 1980; p 215.
Singleton, V. L.; Draper, D. E. The transfer of polyphenolic compounds from grape seeds into wines. *Am. J. Enol. Vitic.* **1964**, *15*, 34.
Singleton, V. L.; Esau, P. Phenolic substances in grapes and wine and their significance. In *Advances in Food Research*; Academic: London, 1969.
Singleton, V. L.; Draper, D. E.; Rossi, J. A. Paper chromatography of phenolic compounds from grapes, particularly seeds and some variety ripeness relationships. *Am. J. Enol. Vitic.* **1966**, *17*, 206.
Stonestreet, E. Contribution à l'étude de la matière colorante des vins rouges. Sc. Doct. Thesis, University of Bordeaux, France, 1965.
Su, C. T.; Singleton, V. L. Identification of three flavan-3-ols from grapes. *Phytochemistry* **1969**, *8*, 1553.
Wilson, E. L. High pressure liquid chromatography of apple juice phenolic compounds. *J. Sci. Food Agric.* **1981**, *32*, 257.

Received for review October 12, 1988. Accepted May 1, 1989.

Formation of Indole Glucosinolate Breakdown Products in Autolyzed, Steamed, and Cooked *Brassica* Vegetables

Bogdan A. Slominski and Lloyd D. Campbell*

The thermal decomposition of indole glucosinolates that occurs during cooking procedures and the autolysis of indole glucosinolates in raw *Brassica* vegetables were studied to evaluate the reported anticarcinogenic properties of these vegetables. Intact indole glucosinolates and the indole glucosinolate breakdown products, thiocyanate ion and indoleacetonitriles, were followed in raw (autolyzed), steamed (10 min), and cooked (40 min) samples of cabbage, cauliflower, broccoli, and Brussels sprouts. Heat treatment resulted in substantial decomposition of indole glucosinolates with thiocyanate ion and indoleacetonitriles accounting for 50 and 30%, respectively, of the degraded indoles. Autolysis, in contrast, resulted in the production of little or no indoleacetonitriles but with considerable production of thiocyanate ion and related compounds (i.e., indolemethanols). The consumption by humans of raw or variously cooked *Brassica* vegetables may result in different intakes of indole glucosinolate derived products, which ultimately could influence the anticarcinogenic properties of the vegetables.

The indole glucosinolates 3-indolylmethyl (glucobrassicin), 4-hydroxy-3-indolylmethyl (4-hydroxyglucobrassicin), and 4-methoxy-3-indolylmethyl (4-methoxyglucobrassicin) represent a significant proportion of the total glucosinolate content of cruciferous vegetables (Fenwick et al., 1983; Truscott et al., 1982). 3-Indolylmethyl glucosinolate has been shown to be the predominant indole glucosinolate in cabbage, broccoli, Brussels sprouts, and cauliflower (Heaney and Fenwick, 1980; Mithen et al., 1987). Virtanen (1965) demonstrated that, following rupture of the cells of plant material, 3-indolylmethyl glucosinolate is hydrolyzed by the endoge-

Department of Animal Science, University of Manitoba, Winnipeg, Manitoba R3T 2N2, Canada.

0021-8561/89/1437-1297$01.50/0

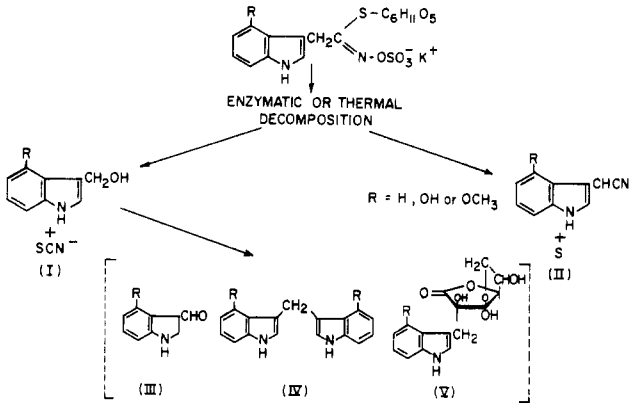

Figure 1. Products of autolytic or thermal degradation of the indole glucosinolates: 3-indolylmethyl (R = H), 4-methoxy-3-indolylmethyl (R = OCH_3), and 4-hydroxy-3-indolylmethyl (R = OH). Decomposed glucosinolates are converted to indolemethanols (I) with the release of free thiocyanate ion or to indoleacetonitriles (II). The formation of aldehydes (III), diindolylmethanes (IV), or ascorbigen (V) from unstable indolemethanols following thermal degradation of indole glucosinolates is hypothetical.

nous enzyme myrosinase (thioglucoside glucohydrolase, EC 3.2.3.1) to a number of compounds including 3-indolemethanol and thiocyanate ion (Figure 1). Both compounds have been shown to be the major product of the autolysis of 3-indolylmethyl glucosinolate, but due to the instability of 3-indolemethanol in aqueous solution the condensation product 3,3′-diindolylmethane and/or the oxidation product 3-indolecarbonaldehyde tend to predominate (Virtanen, 1965; Bradfield and Bjeldanes, 1987). Furthermore, in the presence of ascorbic acid, 3-indolemethanol may also be converted to ascorbigen (Gmelin and Virtanen, 1961; Searle et al., 1984). Although not well documented, it is probable that 4-hydroxy-3-indolylmethyl and 4-methoxy-3-indolylmethyl glucosinolates yield autolytic products analogous to those of 3-indolylmethyl glucosinolate (Figure 1). In addition, it has been well documented that indole glucosinolates are susceptible to thermal degradation (Srisangnam et al., 1980) with the production of breakdown products similar to those observed in autolysis but with a relatively high level of the nitriles 3-indoleacetonitrile and 4-hydroxy-3-indoleacetonitrile (Wall et al., 1988; Slominski and Campbell, 1989; Campbell and Slominski, 1989) (Figure 1).

Recently the consumption of *Brassica* vegetables has been associated with a reduction in the incidence of cancer in humans (National Research Council, 1982). In studies with laboratory animals, indole derivatives, specifically 3-indolemethanol, 3,3′-diindolylmethane, and 3-indoleacetonitrile, have been shown to inhibit forestomach neoplasia and mammary tumor formation and to increase the activity of key enzymes involved in cellular detoxification systems (Wattenberg and Loub, 1978). The results of a number of epidemiological studies and experimental studies of the inhibitory effects of the indole constituents of cruciferous vegetables on carcinogenesis have been summarized by the National Research Council (1982) and by Fenwick and Heaney (1983). In contrast, some of the breakdown products of the glucosinolates, particularly the nitriles, may have antinutritive or toxic properties as reported by Tookey et al. (1980) in studies with seed meal of *Crambe abyssinica*.

Due to the potential for different biological responses to indole derivatives, the current study was conducted to compare the levels of indole glucosinolate breakdown products in autolyzed and heat-treated *Brassica* vegetables. These data will be useful in assessing the intake of indole derivatives among humans consuming raw or variously heat-treated *Brassica* vegetables.

ANALYTICAL METHODS

Individual intact glucosinolates were determined according to the method of Thies as modified by Slominski and Campbell (1987). For the analysis of fresh vegetables, myrosinase enzyme was inactivated by placing the samples (100 mg) contained in a test tube into a boiling water bath for 15 min. Boiling water (2 mL) was added to each test tube, and the heat treatment was continued for an additional 3 min. Cooked cabbage (200 mg) and cooking water (100 mg) samples were prepared for analysis by adding 2 mL of cold water to test tubes containing these samples. Internal standard solution (1 mL, 0.5 μmol mL^{-1} of benzyl glucosinolate; Agriculture Canada, Saskatoon, Canada) and 200 μL of a 1:1 (v/v) mixture of 0.5 M barium acetate and 0.5 M lead acetate were then added to all samples. Following extraction and centrifugation, the resulting supernatant (0.7 mL) was applied to a DEAE Sephadex A-25 column (pyridine acetate form). Sulfatase (aryl-sulfate sulfohydrolase, EC 3.1.6.1; Sigma) solution (50 μL) was added to the column, and the contents were allowed to incubate at room temperature overnight. The resulting desulfated glucosinolates were then eluted with water (4 × 0.5 mL), and following evaporation under nitrogen, the dry residue in the eluent was trimethylsilylated with 100 μL of pyridine, 50 μL of *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide, and 10 μL of trimethylchlorosilane. The trimethylsilyl (TMS) derivatives of desulfoglucosinolates were separated by gas chromatography on a glass column (1.2 m × 2 mm (i.d.)) packed with 2% OV-7 on Chromosorb W (HP) (100–120 mesh) with helium gas at a flow rate of 40 mL min^{-1}. The oven temperature was kept at 200 °C for 4 min and then increased at 5 °C min^{-1} to 275 °C. Injection port and detector temperatures were 280 and 300 °C, respectively. Relative response factors (RRF) were calculated from the ratios of the internal standard TMS carbon number and the respective glucosinolate TMS carbon number. In addition, since aliphatic (allyl, 3-butenyl, 2-hydroxy-3-butenyl), benzyl, and indole (glucobrassicin and 4-hydroxyglucobrassicin) glucosinolates respond differently in the flame ionization detector, corrections were made. The calculated RRF values were corrected by a factor of 0.72 for aliphatic glucosinolates and by a factor of 1.48 for indole glucosinolates. Allyl (Aldrich) and 4-hydroxy-3-indolylmethyl glucosinolates were used along with benzyl glucosinolate (internal standard) to determine the correction factors. For these determinations, 4-hydroxy-3-indolylmethyl glucosinolate was isolated from low-glucosinolate rapeseed and purified according to the method of Slominski and Campbell (1987).

Indoleacetonitriles (3-indoleacetonitrile, and 4-hydroxy-3-indoleacetonitrile) were determined by gas chromatography according to Slominski and Campbell (1988). Samples (0.5 g) of fresh or heat-treated *Brassica* vegetables were extracted for 15 min on a shaker following addition of 5 mL of water, 30 mL of dichloromethane (CH_2Cl_2), and 1 mL of internal standard (0.25 μmol mL^{-1} *n*-octodecane in CH_2Cl_2). The CH_2Cl_2 extracts were then filtered, concentrated under vacuum, and transferred to silylation vials. Following drying under a stream of nitrogen, the samples were derivatized at room temperature by the addition of 50 μL of pyridine and 50 μL of bis(trimethylsilyl)trifluoroacetamide. The derivatized indoleacetonitriles were analyzed by GC using a glass column (1.8-mm i.d.) packed with 3% OV-1 on Chromosorb W (HP) (100–120 mesh) with helium gas at a flow rate of 40 mL min^{-1}. Oven temperature was programmed from 120 to 220 °C at 5 °C min^{-1}, and the temperatures of the injection port and detector were 200 and 250 °C, respectively. The response factors used were 1.58 for 3-indoleacetonitrile and 1.36 for 4-hydroxy-3-indoleacetonitrile.

A modified method of Johnston and Jones (1965) was used for the analysis of free thiocyanate ion in steamed, cooked, and autolyzed *Brassica* vegetable samples. Water (10 mL) was added to each sample (0.5 g), and following extraction for 30 min, 5 mL of 20% TCA was added. The samples were then centrifuged, and 3 mL of supernatant in triplicate lots was mixed with 3 mL of 0.4 M ferric nitrate in 1 N nitric acid. Two drops of 5% mercuric chloride solution were added to one of the tubes (blank sample), and readings were taken for all tubes within 1–2 min at 460 nm.

Table I. Glucosinolate Content of Edible Parts of *Brassica* Vegetables

glucosinolate	cabbage		broccoli		cauliflower		Brussels sprouts	
	A[a]	B[b]	A	B	A	B	A	B
allyl	0.62[c]	25.8	nd[d]	nd	0.10	4.2	1.60	44.0
3-butenyl	0.03	1.3	0.02	0.9	0.01	0.4	0.19	8.2
2-hydroxy-3-butenyl	0.07	3.1	0.20	8.9	nd	nd	0.34	15.1
3-indolylmethyl	0.36	18.2	0.25	12.6	0.13	6.6	1.24	62.7
4-hydroxy-3-indolylmethyl	0.05	2.6	0.10	5.2	0.20	1.0	0.08	4.2
total	1.12	51.0	0.57	27.6	0.27	12.2	2.91	134.2
indole glucosinolates,[e] %		41		64		62		50

[a] Expressed as micromoles per gram fresh weight. [b] Expressed as milligrams/100 g fresh weight. [c] Mean of triplicate determinations. [d] Not detected. [e] Includes 3-indolylmethyl and 4-hydroxy-3-indolylmethyl glucosinolates.

Potassium thiocyanate (J. T. Baker, Phillipsburg, NJ) was used to prepare a standard curve.

The thiocyanate ion method of indole glucosinolate analysis of fresh vegetable samples involved the determination of thiocyanate ion released upon incubation of samples of heat-treated vegetables with myrosinase enzyme. Vegetable samples (0.5 g) were heat-treated with 7 mL of boiling water for 3 min. The samples were cooled, and 3 mL of myrosinase solution (10 mg mL^{-1}) was added. Following incubation for 2 h, 5 mL of 20% TCA was added and thiocyanate ion contents were determined as described for free thiocyanate ion.

SOURCE OF SAMPLES AND ANALYTICAL PROCEDURES

Brassica vegetables were purchased from a local supermarket. The vegetable samples included Brussels sprouts buttons and heads of white cabbage, cauliflower, and broccoli.

The contents of individual intact glucosinolates were determined on fresh samples of all vegetables by GC as described in Analytical Methods. Indole glucosinolate content of each vegetable sample as determined by the GC method was compared with that estimated from the release of thiocyanate ion upon treatment of the samples with myrosinase enzyme (thiocyanate ion method as described in Analytical Methods). The breakdown products of indole glucosinolates produced by autolysis or by thermal decomposition were studied in all four *Brassica* vegetable samples. Raw (autolyzed), steamed (10 min), or cooked (boiled in water for 40 min) samples were analyzed for indoleacetonitriles and thiocyanate ion as described in Analytical Methods. Autolysis was considered to be complete during the extraction times involved in each of the analytical procedures. The rate of thermal degradation of indole glucosinolates and release of thiocyanate ion and/or indoleacetonitriles was studied by subjecting cabbage to heat treatment for varying time periods. Samples of raw cabbage contained in test tubes were placed in a boiling water bath for 15 min, and then boiling water was added to the samples and the heat treatment was continued for 3, 10, 20, 30, 40, and 50 min. Indole glucosinolate, thiocyanate ion, and indoleacetonitrile analyses were conducted as described in Analytical Methods.

The relative amounts of indole glucosinolates and thermal breakdown products in *Brassica* vegetables were studied in cabbage following cooking. Indole glucosinolate, thiocyanate ion, and indoleacetonitrile analyses were conducted as described in Analytical Methods on cooked cabbage and on the cooking water. These values were compared with the indole glucosinolate content of raw (uncooked) cabbage.

RESULTS AND DISCUSSION

The major glucosinolates in cabbage, Brussels sprouts, and cauliflower were identified as allyl (2-propenyl) and 3-indolylmethyl glucosinolates while broccoli contained a predominance of 3-indolylmethyl but no detectable allyl glucosinolate (Table I; Figure 2). The indole glucosinolates (3-indolylmethyl, 4-hydroxy-3-indolylmethyl) represented 41–64% of total glucosinolates in the four vegetable samples, with the highest concentration present in Brussels sprouts (Table I). Similar amounts of indole glucosinolates have been reported in other studies as summarized by Fenwick et al. (1983).

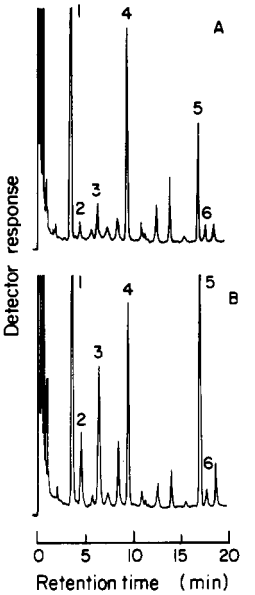

Figure 2. Gas chromatogram of trimethylsilyl derivatives of desulfoglucosinolates from the *Brassica* vegetables (A) cabbage and (B) Brussels sprouts: 1, allyl (sinigrin); 2, 3-butenyl (gluconapin); 3, 2-hydroxy-3-butenyl (progoitrin); 4, benzyl (glucotropaeolin), internal standard; 5, 3-indolylmethyl (glucobrassicin); 6, 4-hydroxy-3-indolylmethyl (hydroxyglucobrassicin).

In the present study the identification of one of the minor indole glucosinolates as 4-hydroxy-3-indolylmethyl glucosinolate was based on similarity of gas chromatograms to those of rapeseed samples (Slominski and Campbell, 1987) and due to the presence of 4-hydroxy-3-indoleacetonitrile in the cooked vegetable samples. Furthermore, positive identification of 4-hydroxy-3-indolylmethyl glucosinolate as a minor component of the indole glucosinolates in cabbage has been reported (Sang et al., 1984). However, due to unavailability of a suitable standard, the presence in the vegetable samples of 4-methoxy-3-indolylmethyl glucosinolate could not be confirmed. This indole glucosinolate was recently identified in cabbage by Truscott et al. (1982). It is probable that a peak representing 4-methoxy-3-indolylmethyl glucosinolate is present on the chromatograms following the peaks of 3-indolylmethyl and 4-hydroxy-3-indolylmethyl glucosinolates (Figure 2). In addition, peaks at retention times between 10 and 15 min may be related to 3-(methylsulfonyl)propyl glucosinolate, which due to instability under chromatographic conditions has been shown to yield a group of peaks (Heaney and Fenwick, 1980). Notwithstanding the unknown peaks in the chromatograms, the data showing correspondence between determinations of indole glucosinolate content by gas chromatography and by the thiocyanate ion method (Table II) indicate a low contribution of 4-methoxy-3-indolylmethyl to the total indole glucosi-

Table II. Indole Glucosinolate[a] Content of *Brassica* Vegetables Determined by Gas Chromatography (GC) or Estimated from Thiocyanate Ion Released following Incubation with Myrosinase Enzyme (SCN Method) (Micromoles per Gram Dry Weight)

vegetable	GC method	SCN method
cabbage	4.34 ± 0.06[b]	5.11 ± 0.04
broccoli	3.56 ± 0.05	4.38 ± 0.02
cauliflower	1.76 ± 0.03	2.66 ± 0.01
Brussels sprouts	8.31 ± 0.03	9.73 ± 0.04

[a] Includes 3-indolylmethyl and 4-hydroxy-3-indolylmethyl glucosinolates. [b] Mean of triplicate determinations ± SD.

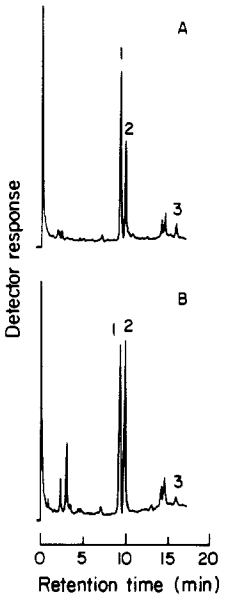

Figure 3. Gas chromatograms of trimethylsilyl derivatives of indoleacetonitriles from the steamed (10 min) *Brassica* vegetables (A) cabbage and (B) Brussel sprouts: 1, *n*-octadecane (internal standard); 2, 3-indoleacetonitrile; 3, 4-hydroxy-3-indoleacetonitrile.

nolate content of the vegetable samples. In this regard, the consistently lower values for the gas chromatography method as compared to the thiocyanate ion method may be due, in part, to some decomposition of indole glucosinolates as a consequence of the heat treatment used to inactivate myrosinase enzyme (Slominski and Campbell, 1987).

Recent studies in our laboratory have demonstrated that, similar to the enzymatic hydrolysis of indole glucosinolates, heat treatment of low-glucosinolate rapeseed meal results in the degradation of 3-indolylmethyl and 4-hydroxy-3-indolylmethyl glucosinolates to yield 3-indoleacetonitrile and 4-hydroxy-3-indoleacetonitrile, respectively (Figure 1) (Slominski and Campbell, 1989). The gas chromatograms shown in Figure 3 indicate the production of these indoleacetonitriles in steamed (10 min) samples of cabbage and Brussels sprouts. In this regard, Wall et al. (1988) reported a major increase in the content of 3-indoleacetonitrile in samples of collards and kale following cooking for 3 or 30 min. In addition to indoleacetonitriles, thiocyanate ion has been shown to be released when *Brassica* crops are subjected to heat treatment (Slominski and Campbell, 1987; Campbell and Slominski, 1989). Thiocyanate ion production indicates that indolemethanols corresponding to the indole glucosinolates present in the heat-treated *Brassica* crop must also be produced (Figure 1). Further conversion to diindolylmethane, indolecarboxaldehyde, or ascorbigen may also occur. In the current study the decomposition of indole glucosinolates in cabbage subjected to heat treatment for

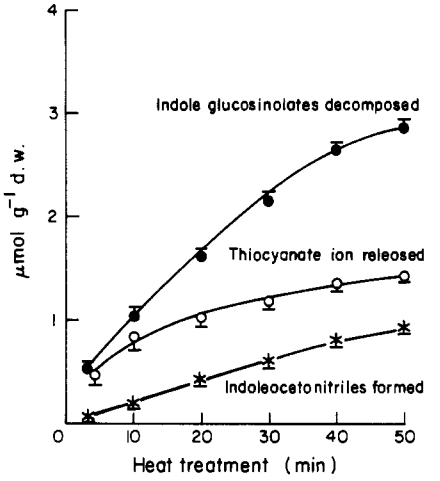

Figure 4. Decomposition of indole glucosinolates (3-indolylmethyl and 4-hydroxy-3-indolylmethyl) and the subsequent release of thiocyanate ion or formation of indoleacetonitriles (3-indoleacetonitrile and 4-hydroxy-3-indoleacetonitrile) in cabbage heated (100 °C) for varying time periods.

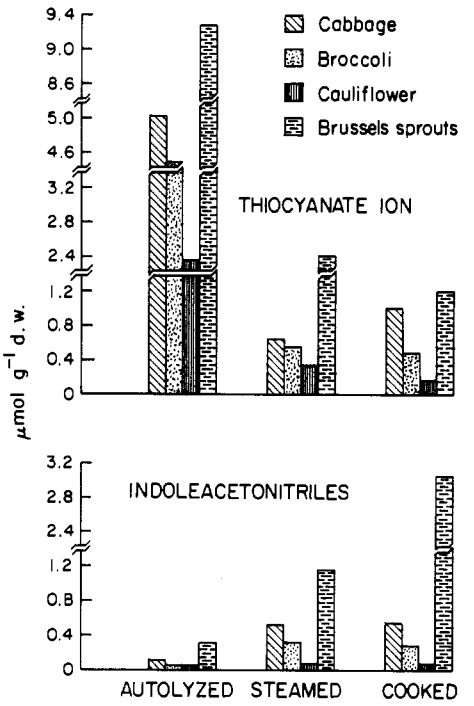

Figure 5. Contents of thiocyanate ion and indoleacetonitriles (3-indoleacetonitrile and 4-hydroxy-3-indoleacetonitrile) in autolyzed, steamed (10 min), or cooked (40 min) *Brassica* vegetables.

varying time periods was quantified as was the release of thiocyanate ion and production of indoleacetonitriles (Figure 4). Since only thiocyanate ion and indoleacetonitriles were monitored, the contents of indolemethanols and related compounds can only be inferred from the thiocyanate ion data. Substantial decomposition occurred within 50 min of heat treatment with thiocyanate ion and indoleacetonitriles accounting for 50 and 30%, respectively, of the degraded indole glucosinolates.

The breakdown products, indoleacetonitriles and thiocyanate ion (indolemethanols and related compounds by inference), were measured in autolyzed, steamed (10 min), and cooked (40 min) *Brassica* vegetable samples (Figure 5). As has been reported for the autolysis of Brussels sprouts (Bradfield and Bjeldanes, 1987) or the autolysis of rapeseed at pH 5–9 (Slominski and Campbell, 1989), the data for raw cabbage, broccoli, cauliflower, and Brussels sprouts indicated a very low production of indoleaceto-

Table III. Recovery of Indole Glucosinolates from Raw Cabbage as Intact Glucosinolates, Thiocyanate Ion, or Indoleacetonitriles in Cooked Cabbage and in the Cooking Water (Micromoles per Gram Dry Weight)

constituent	raw cabbage	cooked cabbage	cooking water	total recovered
intact indole glucosinolates[a]	5.06 ± 0.17	1.12 ± 0.06	1.41 ± 0.07	2.54
thiocyanate ion		0.60 ± 0.04	0.70 ± 0.06	1.30
indoleacetonitriles[b]		0.36 ± 0.01	0.38 ± 0.02	0.74
total		2.08	2.49	4.58

[a] Includes 3-indolylmethyl and 4-hydoxy-3-indolylmethyl glucosinolates. [b] Includes 3-indoleacetonitrile and 4-hydroxy-3-indoleacetonitrile. [c] Mean of triplicate determinations ± SD.

nitriles on autolysis. Thiocyanate ion content of the samples was high, inferring substantial production of indolomethanols and related compounds. In contrast, Wall et al. (1988) reported that autolysis in several cruciferous vegetables produced only traces of 3-indolemethanol. The results of the study by Wall et al. may not reflect true autolysis, however, since the samples were extracted with a ethanol–water (50:50, v/v) solution. The formation of both thiocyanate ion and indoleacetonitriles as a consequence of heat treatment was evident for steamed and cooked samples in the current study. Although the extent of thermal degradation of indole glucosinolates varied among samples of cabbage, broccoli, or cauliflower, the relative amounts of thiocyanate ion and indoleacetonitriles were similar whether the vegetables were heat treated for 10 (steamed) or 40 min (cooked). This may be explained by substantial leaching of the degradation products during the cooking process as opposed to steaming. Brussels sprouts did not show this same pattern possibly due to the fact that whole Brussels sprouts buttons were used. The fact that significant leaching of thiocyanate ion and indoleacetonitriles may occur was confirmed by the analysis of cooked cabbage and cooking water (Table III). Of the intact glucosinolates (5.1 μmol g^{-1} dry weight) present in raw cabbage 90% was recovered in cooked cabbage and in the cooking water as intact glucosinolates, thiocyanate ion, and indoleacetonitriles (2.5, 1.3, and 0.8 μmol g^{-1} dry weight, respectively). In each case cooking water contained over 50% of the glucosinolates or glucosinolate breakdown products.

It can be theorized based on the data presented in this paper that the consumption by humans of raw or cooked *Brassica* vegetables will result in varied intake of indole glucosinolates and their breakdown products. Consumption of raw vegetables will result, as a consequence of autolysis during chewing, in the intake of primarily indolemethanols and related compounds with minimal indoleacetonitriles. The consumption of cooked vegetables, however, will result in intake of both indoleacetonitriles and indolemethanols and related compounds. While cooking of vegetables will produce, in general, greater decomposition of indole glucosinolates than will steaming, the actual consumption of the indole glucosinolate breakdown products may not differ substantially between these two methods of preparation due to considerable leaching of the breakdown products during cooking. On the other hand, a greater quantity of intact glucosinolates will be consumed with steamed as opposed to cooked vegetables. Consumption of vegetables as soups would likely result in a relatively high consumption of breakdown products.

It has been reported that, relative to indoleacetonitriles, indolemethanols are more potent inhibitors of chemically induced mammary tumors in rats (Wattenberg and Loub, 1978) or more effective inducers of aryl hydrocarbon hydroxylase (Loub et al., 1975); consequently, the anticarcinogenic effects of *Brassica* vegetables may differ depending on whether they are consumed raw or cooked. In this regard it seems probable that the inhibitory effects on the development of stomach and colon cancer attributed to *Brassica* vegetables (Graham et al., 1972; 1978; Haensel et al., 1972) should be attributed primarily to the consumption of raw vegetables. Furthermore, it has been reported that the consumption of large amounts of vegetables led to a reduction in the risk of stomach cancer in Norway but not in the United States (National Research Council, 1982). Variations in the way the vegetables were prepared may have contributed to this apparent discrepancy although variations in the contents of indole glucosinolates within and among species and cultivars of vegetables may also have been a factor.

SUMMARY AND CONCLUSIONS

Indole glucosinolates and predominantly glucobrassicin were the major glucosinolates found to be present in the *Brassica* vegetables studied. Heat treatment including steaming and cooking procedures resulted in substantial decomposition of indole glucosinolates with thiocyanate ion and indoleacetonitriles accounting for 50 and 30%, respectively, of the degraded indoles. Autolysis of indole glucosinolates in raw *Brassica* vegetables resulted in the production of little or no indoleacetonitriles but produced substantial thiocyanate ion and related compounds (i.e., indolemethanols). Leaching of intact indole glucosinolates and thermal degradation products of indole glucosinolates occurred during cooking. It may be surmised from the results that the reported anticarcinogenic properties of *Brassica* vegetables will depend on the method of preparation of the consumed vegetable because of the potential for varied intakes of indole glucosinolates and related thermal degradation products.

ACKNOWLEDGMENT

Partial funding provided by the Natural Sciences and Engineering Research Council of Canada is gratefully acknowledged.

LITERATURE CITED

Bradfield, C. A.; Bjeldanes, L. F. High-Performance Liquid Chromatographic Analysis of Anticarcinogenic Indoles in *Brassica oleracea*. *J. Agric. Food. Chem.* **1987**, *35*, 46–49.

Campbell, L. D.; Slominski, B. A. Further Studies on the Sources of Thiocyanate Ion in the Excreta of Poultry Fed Low-glucosinolate Rapeseed Meal. *J. Sci. Food Agric.* **1989**, *47*, 61–73.

Fenwick, G. R.; Heaney, R. K. Glucosinolates and Their Breakdown Products in Cruciferous Crops, Foods and Feedingstuffs. *Food Chem.* **1983**, *II*, 249–271.

Fenwick, G. R.; Heaney, R. K.; Mullin, W. J. Glucosinolates and Their Breakdown Products in Food and Food Plants. *CRC Crit. Rev. Food Sci. Nutr.* **1983**, *18*, 123–201.

Gmelin, R.; Virtanen, A. I. Glucobrassicin, the Precursor of SCN, -3-indolylacetonitrile and Ascorbigen in Brassica Oleracea spp. *Ann. Acad. Sci. Fenn., Ser. A2* **1961**, *No. 107*, 1–25.

Graham, S.; Schote, W.; Martino, P. Alimentary Factors in the Epidemiology of Gastric Cancer. *Cancer* **1972**, *30*, 927–938.

Graham, S.; Dayal, M.; Swanson, A.; Mittelman, A.; Wilkinson, G. Diet in the Epidemiology of Cancer of the Colon and Rectum. *JNCI, J. Natl. Cancer Inst.* **1978**, *51*, 709–714.

Haensel, W.; Kusihana, M.; Sagi, M.; Lee, R. K. C. Stomach Cancer Among Japanese in Hawaii. *JNCI, J. Natl. Cancer Inst.* **1972**, *49*, 969–988.

Heaney, R. K.; Fenwick, G. R. Glucosinolates in Brassica Vegetables. Analysis of 22 Varieties of Brussels Sprout (*Brassica oleracea* var. gemmiferal). *J. Sci. Food Agric.* **1980**, *31*, 785–793.

Johnston, T. D.; Jones, D. I. H. Variation in the Thiocyanate Content of Kale Varieties. *J. Sci. Food Agric.* **1966**, *17*, 70–71.

Loub, W. D.; Wattenberg, L. W.; Davis, D. W. Aryl Hydrocarbon Hydroxylase Induction in Rat Tissues by Naturally Occurring Indoles of Cruciferous Plants. *JNCI, J. Natl. Cancer Inst.* **1975**, *54*, 985–988.

Mithen, R. F.; Lewis, B. G.; Heaney, R. K.; Fenwick, G. R. Glucosinolates of Wild and Cultivated Brassica Species. *Phytochemistry* **1987**, *26*, 1969–1973.

National Research Council. *Diet, Nutrition and Cancer*; National Academy Press: Washington, DC, 1982.

Sang, J. P.; Minchinton, I. R.; Johnstone, P. K.; Truscott, R. J. W. Glucosinolate Profiles in the Seed, Root and Leaf Tissue of Cabbage, Mustard, Rapeseed, Radish and Swede. *Can. J. Plant Sci.* **1984**, *64*, 77–93.

Searle, L. M.; Chamberlain, K.; Butcher, D. N. Preliminary Studies on the Effects of Copper, Iron and Manganese Ions on the Degradation of 3-indolylmethyl Glucosinolate (a constituent of Brassica spp.) by Myrosinase. *J. Sci. Food Agric.* **1984**, *35*, 745–748.

Slominski, B. A.; Campbell, L. D. Gas Chromatographic Determination of Indole Glucosinolates – A Re-examination. *J. Sci. Food Agric.* **1987**, *40*, 131–143.

Slominski, B. A.; Campbell, L. D. Gas Chromatographic Determination of Indoleacetonitriles – Thermal Degradation Products of Indole Glucosinolates in Rapeseed and Brassica Vegetables. *J. Chromatogr.* **1988**, *454*, 285–291.

Slominski, B. A.; Campbell, L. D. Indoleacetonitriles – Thermal Degradation Products of Indole Glucosinolates in Commercial Rapeseed Meal. *J. Sci. Food Agric.* **1989**, *4*, 75–84.

Srisangnam, C.; Salunke, D. K.; Reddy, N. R.; Dull, G. G. Quality of cabbage. II. Physical, Chemical and Biochemical Modification in Processing Treatments to Improve Flavour of Blanched Cabbage (*Brassica oleracea* L.). *J. Food Qual.* **1980**, *3*, 233–239.

Tookey, H. L.; VanEtten, C. H.; Daxenbichler, M. E. Glucosinolates. In *Toxic Constituents of Plant Foodstuffs*; Liener, I. E., Ed.; Academic Press: New York, 1980; pp 103–140.

Truscott, R. J. W.; Minchinton, I. R.; Burke, D. G.; Sang, Y. P. A Novel Methoxyindole Glucosinolate. *Biochem. Biophys. Res. Commun.* **1982**, *107*, 1368–1375.

Virtanen, A. I. Studies on Organic Sulphur Compounds and Other Labile Substances in Plants – A Review. *Phytochemistry* **1965**, *4*, 207–228.

Wall, M. E.; Taylor, H.; Perera, P.; Wani, M. C. Indoles in Edible Members of the Cruciferae. *J. Nat. Prods, Lloydia* **1988**, *51*, 129–135.

Wattenberg, L. W.; Loub, W. D. Inhibition of Polycyclic Aromatic Hydrocarbon-induced Neoplasia by Naturally Occurring Indoles. *Cancer Res.* **1978**, *38*, 1410–1413.

Received for review April 6, 1988. Accepted April 3, 1989.

Novel Approach to the Extraction of Herbicides and Their Metabolites from Plant Tissues[1]

Sherman D. Nelson* and Shahamat U. Khan

A pressure extraction technique was developed and used to extract the herbicide atrazine and its metabolites from plant tissues of bean, soybean, and canola. Single leaves were placed in a pressure chamber, and plant fluids were expressed under 0–6.2 MPa of applied pressure through and collected from the leaf vascular system. Up to 98% of the total tissue fluid was extracted, and the procedure provided "clean" samples that were injected directly into an HPLC for analysis. Pressure–concentration release curves for atrazine and metabolites, and freeze–thaw treatment to disrupt membrane integrity, allowed interpretation of herbicide compartmentalization and metabolism within tissues. The pressure extraction procedure should prove to be very useful in the study of plant–herbicide relations.

The "Scholander-Hammel" pressure chamber has been extensively used to determine a variety of plant water relations parameters including tissue osmotic potential, tissue water potential, cell wall bulk modulus of elasticity, and amounts of bound and osmotically active water (Ritchie and Hinckley, 1975; Tyree et al., 1973; Hellkivist et al., 1974). Stroshine et al. (1979) has used the technique to model water movement in wheat leaves. The expression of tissue fluid by applied pressure has also been used to collect sap for solute analysis; Ackerson (1982) collected fluid for abscisic acid analysis, and Hartung et al. (1988) investigated absicisic acive movement in water-stressed cotton leaves. Recently, Jachetta et al. (1986a) have used a "pressure dehydration" technique to deduce the origin of expressed fluid. They distinguished three origins of sap as pressure was increased over small, 0.02–0.04 MPa, pressure intervals between 0.0 and 0.5 MPa. These sap fractions were sequentially released, and their origins were petiole–main vien fraction, minor vien–cell wall fraction, and a mixed fraction comprised of a decreasing minor vien–cell wall component containing increasing amounts of plasma membrane filtered symplastic fluid. Further studies by Jachetta et al. (1986b) determined the transport and distribution pattern of shoot-fed herbicides, atrazine and glyphosate, within detached sunflower leaves. Their results confirmed that atrazine movement followed the apoplastic pattern (Ashton and Crafts, 1973) whereas glyphosate movement was mainly via a symplastic pattern (Dewey and Appleby, 1983; Gougler and Geiger, 1981). Jachetta et al. (1986a) indicated the potential usefulness of the pressure dehydration method for the study of sub-

Land Resource Research Centre, Agriculture Canada, Central Experimental Farm, Ottawa, Ontario, Canada K1A 0C6.

[1] LRRC Contribution No. 88-53.

0021-8561/89/1437-1302$01.50/0 Published 1989 by the American Chemical Society