

Glucosinolates and Derived Products in Cruciferous Vegetables. Analysis of 14 Varieties of Chinese Cabbage

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The glucosinolate compositions of 14 varieties of Chinese cabbage (*Brassica campestris* ssp. *pekinensis*) are reported and contrasted to those previously reported for common cabbage (*B. oleracea* var. *capitata*). Glucosinolates yielding five-carbon aglucons (excluding the sinolate carbon) predominate in the Chinese cabbage, whereas three- and four-carbon aglucons are the major ones from white and red cabbage varieties, respectively, that were reported earlier. The range of total glucosinolates was similar in both (174–1357 ppm for Chinese and 299–1288 ppm for other cabbage). Seed from 13 of these varieties of Chinese cabbage was analyzed. The major glucosinolate in the seed is 3-butenyl glucosinolate.

As part of a larger program to study natural toxicants, we are making extensive measurements of the kinds and amounts of glucosinolates present in cruciferous vegetables and their seed. We recently reported a summary of our measurements for these compounds in 22 varieties of cabbage (*Brassica oleracea* var. *capitata*) (VanEtten et al., 1976; VanEtten and Daxenbichler, 1977; Daxenbichler and VanEtten, 1977). We did not include measurements for the Chinese cabbage varieties in that report because they constitute an altogether different species (*Brassica campestris* ssp. *pekinensis*) and accordingly their composition is vastly different from that of common cabbage (Nieuwhof, 1969). The glucosinolates and their hydrolytic products contribute to the flavor of these vegetables and are toxicants that could, if consumed in large quantity, be harmful (VanEtten and Wolff, 1973). Reported here are detailed measurements of the kinds and amounts of glucosinolates in the fresh parts and seed from 14 varieties of Chinese cabbage. Such data will assist in evaluation of the significance relative to glucosinolate content that may be considered in the development and release of new cultivars.

EXPERIMENTAL SECTION

Sample Source and Preparation. The varieties Michihili (Northrup King Co.) and F₁ Hybrid G (Harris Co.) were planted in 1975 on adjacent plots at the University of Wisconsin, Madison. The early crop was planted on May 15 and harvested on July 24; the late crop was planted on August 7 and harvested on October 14. Three to five typical heads were selected for analyses. In 1976, a total of 14 varieties were grown at this same location. They were planted on May 14 and harvested on August 5. Except during shipment, the heads were refrigerated until sampled for analysis (less than 30 days). From each head 100 g was taken as three slices at right angles to the direction of growth. One slice was taken near the bottom, a second in the center, and a third near the top. Extracts from the 100-g head samples were prepared as previously described (VanEtten et al., 1976).

Seed samples from 13 of the varieties grown in 1976 were ground and defatted with petroleum ether (pentane-hexane) before analysis. For analysis, 250-mg defatted meal samples were extracted with 15 mL of boiling water,

followed by filtration and reextraction (4 × 15 mL) to make a volume of about 75 mL.

Methods of Analysis. Total glucosinolates in the heads and seed meals were determined from enzymatically liberated glucose following an ion-exchange separation of the glucosinolates from most of the extraneous materials (VanEtten and Daxenbichler, 1977). Measurements representing the specific glucosinolates present were obtained by gas-liquid chromatography (GLC) of the aglucon hydrolytic products obtained as part of the preparation for the glucose release measurement (Daxenbichler and VanEtten, 1977) and by estimation in a separate aliquot of thiocyanate ion (SCN) which represents the 3-indolylmethyl glucosinolates (VanEtten et al., 1976) (Scheme I).

Identification and Quantitation of the Aglucons. Preparations of the aglucons from 2 of the 14 varieties were subjected to gas chromatography-mass spectrometry (GC-MS) study. Accordingly, peaks for the respective components in the other varieties were assigned by comparison of retention times on the two GLC columns (Daxenbichler and VanEtten, 1977). The two varieties used in the GC-MS study were Kyoto No. 3 (Takii) and Michihili (Northrup King). These two were also the most widely separated when the varieties were grouped by statistical comparison of the glucosinolate composition (Table I). The GC-MS tandem system used was as described by Spencer et al. (1976). Three components found in Chinese cabbage were not listed in our GLC analytical method for common cabbage (Daxenbichler and VanEtten, 1977). Under the conditions previously described, 5-methylthiopentyl isothiocyanate (5-methylthiopentyl-NCS) emerged from the Apiezon column at about 30 min, i.e., between phenylethyl-NCS and 5-vinylloxazolidine-2-thione (5-vinyl-OZT), and about 20 min, midway between phenylethyl-NCS and methyl palmitate on the EGSS-X column. The 5-methylsulfinylpentyl-NCS eluted from the Apiezon column and the EGSS-X column at nearly the same time (at about 43 min), soon after emergence of methyl palmitate on the Apiezon column. When 5-allyl-OZT was detected, it emerged about 2–3 min after 5-vinyl-OZT on the Apiezon column and also just following and well resolved from 5-vinyl-OZT on the EGSS-X column. On the latter column, however, the 5-allyl-OZT tended to elute with the 5-methylsulfinylpentyl-NCS component if they occurred together.

For quantitation of the GLC peaks, the same procedure and response factors were used as previously described (Daxenbichler and VanEtten, 1977). For the additional components found in Chinese cabbage, we used the following detector response factors to normalize the peak

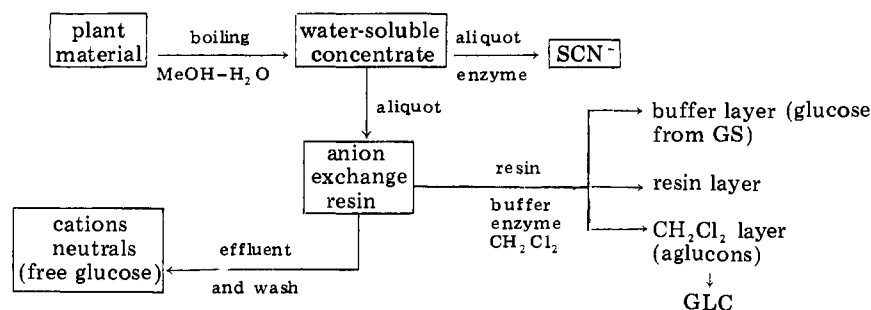
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Table I. Aglucons and Total Glucosinolate from Edible Part of 14 Varieties of Chinese Cabbage

name and sampling	seed company source	F ₁ ^a or OP	no. of heads	av. head wt, kg	4-carbon aglucons			5-carbon aglucons				phenyl-ethyl-NCS, ppm	indolyl type as SCN ion, ppm	total glucosionate, b ppm	glucose calcd from aglucons, % ^c
					3-butenyl-NCS, ppm	vinyl OZT, ppm	4-pentenyl-NCS, ppm	5-thio-pentyl-NCS, ppm	5-methyl-sulfinyl-pentyl-NCS, ppm	allyl-OZT, ppm					
											12.7				
Michihili (early harvest 7-24-75)	Northrup-King	OP	5	0.3	12.7	25.4	50.9	30.4	100.5	6.1	28.7	50.4	1201	99	
Michihili (late harvest 10-14-75)	Northrup-King	OP	3	0.8	2.2	7.6	16.5	11.1	66.4	2.2	22.6	19.7	545	96	
Michihili (1976)	Northrup-King	OP	1 ^d		0.9	1.7	5.3	7.0	18.9	0.0	20.9	21.2	305	102	
Kurihara	Takii	OP	3	0.9	3.7	7.9	4.7	7.8	8.9	1.0	17.1	16.7	289	99	
WR Super 80	Takii	F ₁	3	1.1	2.8	0.9	4.4	1.5	1.6	0.1	13.6	18.3	174	125	
Hybrid G (1976)	Harris	F ₁	3		1.0	2.4	7.2	3.0	3.3	3.4	13.1	19.3	239	106	
Hybrid G (early harvest 7-21-75)	Harris	F ₁	5	0.2	13.2	3.7	44.1	4.9	21.8	11.0	7.3	63.5	811	100	
Nagaoka King	Sakata	F ₁	3	1.0	1.1	3.2	20.2	0.7	6.1	9.4	13.6	16.0	272	111	
Hybrid G (late harvest 10-21-75)	Harris	F ₁	3	1.1	2.2	2.4	16.5	0.4	10.6	3.0	6.3	15.7	253	100	
Chee Hoo	Takii	OP	2	0.6	17.0	0.8	25.2	0.5	13.1	1.5	11.3	26.0	371	130	
Winter Giant	Sakata	F ₁	1 ^e		17.3	31.7	82.2	4.6	15.3	19.0	28.9	37.0	922	107	
Tip Top No. 12	Sakata	F ₁	3	1.4	15.2	5.9	31.1	9.2	18.8	1.5	13.2	18.0	422	107	
Chitose Giant	Takii	OP	3	1.8	19.0	5.2	39.8	3.0	9.9	0.0	17.6	12.7	422	103	
Tokyo Giant	Takii	OP	3	1.3	31.2	5.9	33.9	2.6	24.7	0.4	21.8	11.3	480	100	
Tetra Nozaki	Takii	OP	3	0.7	47.8	4.4	41.7	5.6	10.5	0.0	19.0	20.5	573	106	
Spring Giant	Sakata	OP	1 ^f	1.7	68.5	7.0	24.6	35.7	53.7	0.1	16.8	20.3	721	116	
Nozaki Early	Takii	OP	2	0.6	3.0	27.2	3.8	5.8	5.5	7.5	9.3	18.0	383	88	
Kyoto No. 3	Takii	OP	1 ^f	0.3	4.2	58.6	22.4	64.0	59.0	17.5	91.0	27.5	1357	88	
mean					14.6	11.2	26.4	11.0	24.9	4.2	20.7	24.0	541	104.6	
rel SD, %					148	98	121	173	176	723	54	20	44		
least significant ratio (3, 3)					4.54	3.13	3.76	5.32	5.44	33.5	2.06	1.35	1.84		

^a F₁ denotes hybrid; OP denotes open pollinated. ^b Calculated from glucose measurement and an average molecular weight of 461 for glucosinolates. ^c Percent relative to glucose obtained as a measure of total glucosinolate. ^d Sampled three times from one head. ^e Sampled one time from one head. ^f Sampled two times from one head.

Scheme I. Outline of Analysis for Glucosinolates



areas relative to the weighed amount of added methyl palmitate as internal standard: 5-methylthiopentyl-NCS, 2.0; 5-methylsulfanyl-pentyl-NCS, 3.0; and allyl-OZT, 2.5. Although the response factors for these components were empirically assigned, they are values similar to those that were experimentally obtained for closely related compounds. The good agreement between the total glucosinolate value determined by the amount of glucose released and the summation calculated from the specific components measured (last column, Table I) lends confidence to the validity of the measurements.

Mass spectra obtained for the various component aglucons from Chinese cabbage agreed with those we earlier obtained for the common cabbages or with authentic samples from other sources (Daxenbichler and VanEtten, 1977). The additional MS obtained from Chinese cabbage for 5-methylthiopentyl-NCS and 5-methylsulfanyl-pentyl-NCS were easily recognized as spectra of members of homologous series when compared with those previously identified. The MS of 5-methylthiopentyl-NCS also agreed with that previously reported by Kjaer et al. (1963). The spectrum for 5-allyl-OZT was identical with that obtained from an authentic sample supplied by B. A. Tapper, who originally described the compound (Tapper and MacGibbon, 1967). A compilation of mass spectral data for aglucon products (nitriles, isothiocyanates, and oxazolidinethiones) derived from the many *Brassica* vegetables and seed that we have studied is currently in preparation. Under certain conditions nitriles and epithio compounds are formed as well as isothiocyanates (Daxenbichler et al., 1977). Little doubt existed as to the identity of the 4-pentenyl-NCS component, because its spectrum agreed with that reported earlier (Kjaer et al., 1963). The spectrum was unusual in that it included a prominent fragment of one mass unit less than the parent ion, which prompted us to isolate a small amount of 4-pentenyl-NCS for verification by nuclear magnetic resonance (NMR). For the isolation, the final dichloromethane solutions of aglucons obtained following ion exchange and enzymatic hydrolysis (equivalent to 400 g of preparations from the varieties Michihili and Hybrid G) were combined, carefully concentrated, and chromatographed on a silica gel column (1 × 20 cm) with ether as eluant. The component eluted in the first few milliliters along with minor amounts of other compounds. After concentration, the partially purified material was applied as a band on a commercially prepared 2-mm silica gel plate (EM Reagents Precoated PLC Plate Silica Gel 60 F-254). A separation was obtained with the solvent system hexane-ether (9:1) and 10-cm migration of solvent front. Several bands were located with a short-wave UV lamp. The band located at R_f 0.65–0.70 was eluted from the adsorbent with small volumes of dichloromethane. By GLC the material isolated (about 2 mg) was shown to be essentially pure 4-pentenyl-NCS with a small amount of

Table II. Summary of Aglucon and Glucosinolate Content of Seed from 13 Varieties of Chinese Cabbage^a

component	mean, %	extremes, %	rel SD, ^d %
3-butenyl-NCS	0.57	0.26–0.80	31
5-vinyl-OZT	0.02	0.01–0.05	62
4-pentenyl-NCS	0.17	0.08–0.50	66
2-phenylethyl-NCS	0.02	0.01–0.03	37
indolyl- aglucons as SCN ion	0.05	0.04–0.07	19
total glucosinolates ^b	3.40	2.30–4.27	21
glucose from aglucons ^c	88.1	68–102	9.5

^a From varieties listed in Table I except F₁ Winter Giant. Computed on air-dried defatted seed. ^b Calculated from glucose measurement as 3-butenylglucosinolate, $M_r = 411$. ^c Percent compared to total glucosinolate measured by glucose. ^d Standard deviation.

3-butenyl-NCS as contaminant. The NMR spectrum confirmed the structure of the compound as 4-pentenyl-NCS.

RESULTS AND DISCUSSION

Kind of Glucosinolates in Chinese Cabbage. "White" cabbage typically contains glucosinolates that yield predominately three-carbon aglucons and "red" cabbage shows much larger amounts of four-carbon aglucons (VanEtten et al., 1976). Data presented in Table I for Chinese cabbage show a preponderance of five-carbon aglucons. Data for the seed, however, do not show the same preponderance of the five-carbon aglucons as was observed for the vegetable. Rather, as shown in Table II, the major glucosinolate in the seed is typically 3-butenyl glucosinolate. With the exception of 4-pentenyl-NCS, the five-carbon aglucons do not occur in the seed in large enough relative amounts to be noted and measured. Minor peaks that occur with some regularity in the GLC analyses of the vegetable are not included in Table I because they usually were such small amounts (0–5 ppm). Four peaks of this type were noted: (1) 4-methylthiobutyl-NCS and 4-methylthio-3-butenyl-NCS mixture, (2) benzyl-NCS, (3) unknown at retention time 5–6 min Apiezon column, and (4) unknown at retention time 10 min Apiezon column. The first peak was shown to be the assigned mixture by GC-MS of variety Kyoto No. 3. This occurrence is surprising because previously 4-methylthio-3-butenyl-NCS was known only in vegetative portions of radish (Friis and Kjaer, 1966).

Glucosinolate Content vs. Variety. In Table I, the varieties are arranged in an order such that those containing similar kinds of glucosinolates are listed together. This arrangement was based on correlations found as to the kind and relative amounts of each of the nine glucosinolates in the varieties. Although the concentration of glucosinolates in the 1975 cabbages (O. P. Michihili and

F₁ Hybrid G) changed sharply with time of harvest, the pattern (relative amounts of each) was similar. For this reason, these harvests were placed closely together in the table. Since most of the varietal data represent three heads, the least significant ratio for triplicate analyses at the bottom of the table may be used to show differences. A statistical study showed a greater variation in glucosinolate concentration from head to head within open-pollinated varieties than within hybrids. This was also true with common cabbage, *B. oleracea* (VanEtten et al., 1976).

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γ Irradiation of Subtropical Fruits. 1. Compositional Tables of Mango, Papaya, Strawberry, and Litchi Fruits at the Edible-Ripe Stage

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Total sugar, protein, and lipid contents of the edible portions of four mango, two papaya, one litchi, and two strawberry cultivars were determined as well as certain vitamins and trace elements. Both control (unirradiated) and fruits irradiated at the commercially recommended doses were analyzed. The chemical composition of the four species of fruit examined was very similar. Statistical analyses of the constituents of the irradiated and nonirradiated fruit showed no difference between them.

Papayas, mangoes, litchis, and strawberries suffer from severe shelf-life problems as a result of postharvest diseases, premature ripening, and insect infestation. The preservation of foodstuffs by ionizing radiation has received much attention over the past few decades and, in South Africa, detailed investigations have been undertaken into the possible use of this process for solving the problems encountered with these fruits (Brodrick et al., 1976, 1977; Thomas and Brodrick, 1977).

One obstacle which thus far is retarding rapid commercialization is the problem of safety for consumption. The work of the International Food Irradiation Project (IFIP) in evaluating the wholesomeness of irradiated foods is directed at answering this question and the positive result of the Expert Committee Meeting convened by WHO/IAEA/FAO (WHO, 1976) in September 1976 is a large measure of the success of the IFIP to date. At this Expert Meeting, following the scrutinizing of detailed, multigeneration, and multispecies feeding studies, two of

the above-mentioned fruits, viz. papayas and strawberries, were recommended for unconditional clearance for public consumption. However, in view of the exceedingly high cost and effort involved in executing such feeding studies, it is unreasonable to expect investigations of similar magnitude to be carried out in each and every fruit considered for radiation treatment. Indeed, the WHO has endorsed the principle: "when in-depth toxicological, nutritional, microbiological and chemical data are collected on a representative food of a given diet class, limited data would suffice for evaluating the safety of such irradiation processes for other foods within the same class". In this context it is important to note that classification of foods into diet classes is by chemical, and not botanical, similarity. Accordingly, due to the apparent chemical similarity between mangoes, papayas, and strawberries, the IFIP has commissioned only a limited feeding study on mangoes, using one species (rats) and over a period of 2 years. However, it was recommended by the IFIP that the results of these studies be complemented by chemical analyses confirming chemical similarity to the two fruits studied in detail. As far as is known, no feeding studies have been carried out on litchis and none are contemplated.

Although compositional observations on the fruits in question are available, much of the information has been

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