Glucosinolates in Chinese Cabbage[a]

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The glucosinolate composition of nine open pollinated and five F_1 hybrid cultivars of Chinese cabbage were determined quantitatively using gas chromatography and mass spectrometry. Glucosinolates yielding 5-carbon aglucons predominate in Chinese cabbage head tissue whereas the 4-carbon, 3-butenyl-isothiocyanate predominates in the seed. Total glucosinolates in head tissue range from 229-1288 ppm. Glucosinolates in Chinese cabbage differ from common cabbage, Brassica oleracea *var* capitata, *which contain 3- and 4-carbon glucosinolates.*

GLUCOSINOLATES

An increasingly important aspect of cruciferous vegetable breeding that has long been recognized by oilseed and forage crucifer breeders is the need to examine the chemical constituents of the crucifer cultivars and breeding lines as an important component of cultivar and crop phenotype. The crucifers are known to be a rich source of the glucosinolates, a class of compounds containing glucose and sulphur whose enzyme breakdown products are known for their pungency, their biological activity in pest attraction and disease resistance, and their role in certain mammalian metabolic disorders (Van Etten and Wolff 1973). Glucosinolates are characterized by the structure depicted in Figure 1, and are differentiated by the chemical structure of their aglucon (R) grouping. About 80 different glucosinolates have been isolated from a wide range of species in the Cruciferae. All crucifers examined have been found to contain one or more glucosinolates. Upon disruption of crucifer cells, thioglucosidase glucohydrolase, (EC 3.2.3.1), commonly known as thioglucosidase or myrosinase, acts hydrolytically upon glucosinolates to release glucose and sulphate ions. The aglucon is usually converted to isothiocyanate through a Lossen rearrangement, to nitriles or to thiocyanate (Figure 1).

Much of the characteristic flavor of Chinese cabbage comes from the isothiocyanates or so-called mustard oils derived from the glucosinolates. Allylisothiocyanate is the volatile mustard oil responsible for the sharp or pungent flavor in condiment mustards *Brassica nigra* and *B. juncea*, and is also responsible for much of the mustard-like components or flavor in common cabbage, collards, kales and mustard greens (Van Etten and Wolff 1973). Studies of the edible parts of common cabbages by Van Etten *et al* (1976) have identified and quantified 11 common glucosinolates (GS) of which 3-methysulfinyl-propyl GS (glucoiberin), 4-methysulfinyl-butyl GS (glucoraphanin),

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allyl GS (sinigrin) and two 3-indolymethyl GS (glucobrassicin and neoglucobrassicin) were the major components. An important glucosinolate found in many *Brassica* species is (R) -2-hydroxy-3-butenyl GS or progoitrin. The isothiocyanate of progoitrin undergoes cyclization to form 5-vinyloxazolidine-2-thione or goitrin. Under certain conditions of enzymatic hydrolysis progoitrin may form an unsaturated nitrile and two diasteriomeric epithio nitriles instead of goitrins (Figure 2). Other important glucosinolates are the indolylmethyl GS, glucobrassicin and neoglucobrassicin which upon hydrolysis yield 3-hydroxymethylindoles and thiocyanate ion. The 3-hydroxymethylindoles may react with ascorbic acid to form ascorbigen. The indolymethylglucosinolates have also been implicated in the biosynthesis of the plant growth hormone indolylacetic acid via indolylacetonitrile (Butcher *et al* 1974).

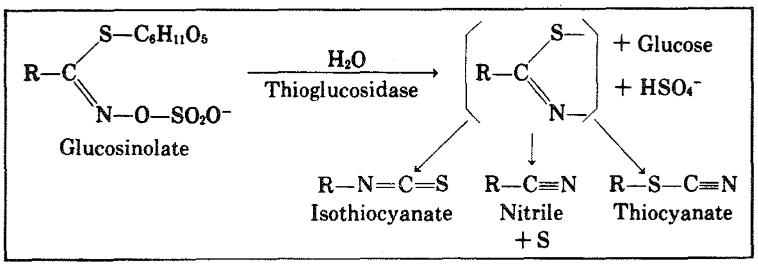

Figure 1. Reaction products of thioglucosidase acting upon a glucosinolate

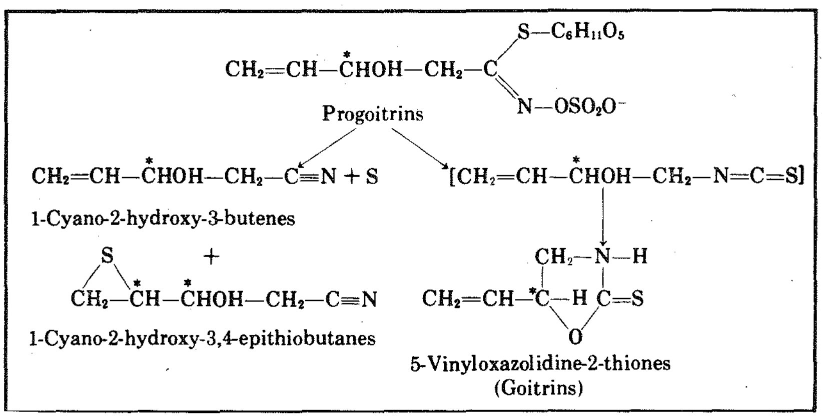

Figure 2. Enzyme hydrolysis products of progoitrin

Goitrin together with allylisothiocyanate and thiocyanate ion have been shown to have goitrogenic activity in test animals. Among the anti-thyroid agents found in the cruciferae, goitrins are the most active (Van Etten and Wolff 1973). Progoitrin is considered to be a minor component of the glucosinolates of most *Brassica* species with the exception of *B. napus*, where it is a major component of both rutubaga roots and oilseed meal.

GLUCOSINOLATES IN CHINESE CABBAGE

As part of a larger program to study natural toxicants in cruciferous vegetables we have recently completed an analysis of 22 cultivars of common cabbage (*B. oleracea* var *capitata*) (Van Etten *et al* 1976) and of 14 Chinese cabbage cultivars (Daxenbichler *et al* 1979). Reported in this presentation will be a summary of the basic information derived from our study on Chinese cabbage.

EXPERIMENTAL SECTION

SAMPLE SOURCE AND PREPARATION

The cultivars Michihili (Northrup King) and F_1 Hybrid-G (Harris Company) were planted in 1975 on adjacent plots at the University of Wisconsin, Madison. The early crop was planted on 15 May and harvested on 24 July; the late crop was planted on 7 August and harvested on 14 October. Three to five typical heads were selected for analysis. In 1976 a total of 14 cultivars were grown at this same location. They were planted on 14 May and harvested on 5 August. Except during shipment, the heads were refrigerated until sampled for analysis (<30 days). From each head one 100 g sample 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 described (Van Etten *et al* 1976).

Seed samples from 13 of the cultivars grown in 1976 were ground and defatted in 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 re-extraction (4 x 15 ml) to make a volume of about 75 ml.

METHOD 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 (Van Etten 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. An estimation of thiocyanate ion (SCN) which represents the 3-indolylmethyl-glucosinolates (Van Etten *et al* 1976) was made in a separate aliquot (Figure 3). Complete details of the analysis are given by Daxenbichler *et al* (1979).

RESULTS AND DISCUSSION

KINDS OF GLUCOSINOLATES

Common cabbage contains glucosinolates that yield predominantly three-carbon aglucons and red cabbage shows much larger amounts of four-carbon aglucons (Van Etten *et al* 1976). Data presented in Table 1 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 vegetative heads. Rather, as is shown in Table 2, 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.

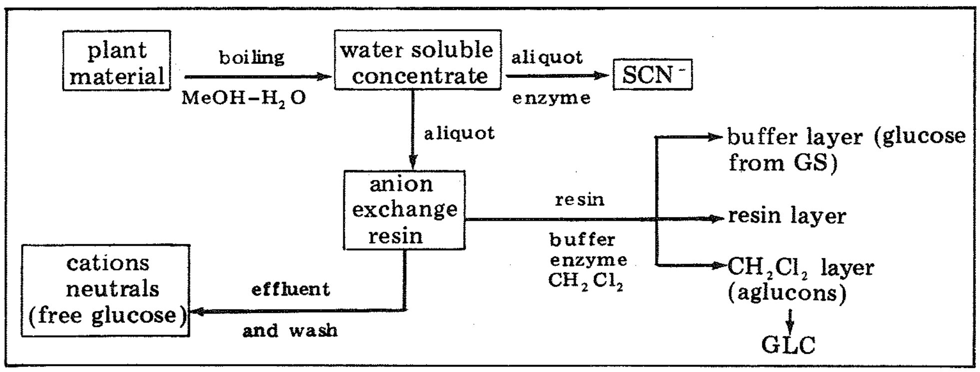

Figure 3. Outline of analysis for glucosinolates

GLUCOSINOLATE CONTENT VS CULTIVAR

In Table 1 the cultivars 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 cultivars. Although the concentration of glucosinolates in the 1975 cabbages (Michihili and 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 tables. Since most of the cultivar 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 cultivars than within hybrids. This was also true with common cabbage (*B. oleracea*) (Van Etten *et al* 1976).

Though at this time only a beginning has been made toward investigating the glucosinolates in Chinese cabbage, the improved quantitative and qualitative methods developed by Daxenbichler and Van Etten (1977) and Daxenbichler *et al* (1977) for glucosinolate analysis of plant tissue will be of considerable value in providing important base line information on the levels and variation of potentially useful and hazardous glucosinolates. Preparative techniques for glucosinolates will also permit biological and toxicological evaluation of specific compounds. Despite the fact that much concern over glucosinolates has been generated on the basis of largely unsubstantiated evidence that the consumption of excessive amounts of *Brassica* crops has resulted in benign goiter (Anon 1974), there is good reason to believe that more information about the heritability and genetics of glucosinolates in Chinese cabbage may be of considerable use to breeders in the future.

Table 1. Aglucons and total glucosinolate from edible parts of 14 cultivars of Chinese cabbage

Cultivar and Source	4-carbon aglucons (ppm)		5-carbon aglucons (ppm)						
	3-butenyl-NCS[a]	vinyl OZT,[b]	4-pentenyl-NCS,	5-methylthiopentyl-NCS,	5-methylsulfinylpentyl-NCS,	allyl-OZT,	phenylethyl-NCS,	indolyl type as SCN ion,	total glucosinolate,[c]
Michihili (early '75) NK, OP[d]	12.7	25.4	50.9	30.4	100.5	6.1	28.7	50.4	1201
Michihili (late '75) NK, OP	2.2	7.6	16.5	11.1	66.4	2.2	22.6	19.7	545
Michihili (1976) NK, OP	0.9	1.7	5.3	7.0	18.9	0.0	20.9	21.2	305
Kurihara, T, OP	3.7	7.9	4.7	7.8	8.9	1.0	17.1	16.7	289
WR Super 80, T, F_1	2.8	0.9	4.4	1.5	1.6	0.1	13.6	18.3	174
Hybrid G (1976) H, F_1	1.0	2.4	7.2	3.0	3.3	3.4	13.1	19.3	239
Hybrid G (early '75) H, F_1	13.2	3.7	44.1	4.9	21.8	11.0	7.3	63.5	811
Nagaoka King S, F_1	1.1	3.2	20.2	0.7	6.1	9.4	13.6	16.0	272
Hybrid G (late '75) H, F_1	2.2	2.4	16.5	0.4	10.6	3.0	6.3	15.7	253
Chee Hoo, T, OP	17.0	0.8	25.2	0.5	13.1	1.5	11.3	26.0	371
Winter Giant, S, F_1	17.3	31.7	82.2	4.6	15.3	19.0	28.9	37.0	922
Tip Top No. 12, S, F_1	15.2	5.9	31.1	9.2	18.8	1.5	13.2	18.0	422
Chitose Giant, T, OP	19.0	5.2	39.8	3.0	9.9	0.0	17.6	12.7	422
Tokyo Giant, S, OP	31.2	5.9	33.9	2.6	24.7	0.4	21.8	11.3	480
Tetra Nozaki, T, OP	47.8	4.4	41.7	5.6	10.5	0.0	19.0	20.5	573
Spring Giant, S, OP	68.5	7.0	24.6	35.7	53.7	0.1	16.8	20.3	721
Nozaki Early, T, OP	3.0	27.2	3.8	5.8	5.5	7.5	9.3	18.0	383
Kyoto No. 3, T, OP	4.2	58.6	22.4	64.0	59.0	17.5	91.0	27.5	1357
mean	14.6	11.2	26.4	11.0	24.9	4.2	20.7	24.0	541
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]NCS= isothiocyanate; [b]OZT= oxazolidine-2-thione; [c]calculated from glucose measurement and an average molecular weight of 461 for glucosinolates; [d]F_1 denotes hybrid; OP denotes open pollinated; NK= Northrup King; H= Harris; S= Sakata; T= Takii Seed Co

By understanding the relationship of flavor to the composition and content of glucosinolates, Chinese cabbage breeders with appropriate knowledge of the genetics of glucosinolates may be able to develop cultivars of enhanced quality (MacLeod 1976). Reports on the role of benzylisothiocyanate and phenylethyl isothiocyanate in inhibiting carcinogin-induced neoplasia in rats and mice through the stimulation of mixed function oxidases (Wattenberg 1978) point to the possibility of introducing into our diets levels of potentially therapeutic chemical constituents. The beneficial effects of crucifer residues incorporated in soils in reducing the severity of pea root rot (*Aphanomyces euteiches*) has been attributed to the fungitoxic activity of allylisothiocyanate and other sulphur-containing breakdown products (Lewis and Papavizas 1971). The insecticidal activity of 2-phenylethyl isothiocyanate from *B. oleracea* (Lichtenstein *et al* 1964) might be more effectively exploited if cultivars containing high levels of the chemical were developed. As Chinese cabbage breeders begin to exploit the diversity within *B. campestris* and *Raphanus* spp through interspecific and intergeneric hybridization (Williams and Heyn 1981), there will be an increasing need to monitor the chemical phenotypes of their resulting crosses. Until now the chemical constituents of most cultivars within each vegetable fall within the spectrum that is representative for that species. As wider crosses are made the resultant progenies are likely to carry vastly different arrays of glucosinolates than their progenitors. Precisely how these new patterns will be expressed in terms of flavor, palatability, toxicity and biological activity remains to be seen.

Table 2. Summary of aglucon and glucosinolate content of seed from 13 cultivars 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]	88.10	68-102	9.5

[a]From cultivars listed in Table 1 except F_1 Winter Giant, computed on air dried defatted seed; [b]calculated from glucose measurement as 3-butyl-glucosinolate, M_r = 411; [c]percent compared to total glucosinolate measured by glucose; [d]standard deviation

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DISCUSSION

J. J. RILEY: Has the glucosinolates content of turnip and pak-choi been analyzed? Is their content of glucosinolates similar to that of Chinese cabbage?

P. H. WILLIAMS: We have just completed the analysis of about 25 turnip materials and the data are presently being tabulated. We have analyzed only a few pak-choi materials as they were not part of our early study. Now we are planning to analyze more cultivars. In fact, we plan on doing a comprehensive survey of oriental types.

B. A. KRATKY: Regarding your comments on goiter and cancer, are you aware of any country with a high consumption of Chinese cabbage that has high goiter and low cancer incidence?

P. H. WILLIAMS: Although I am not familiar with the statistics for goiter or cancer epidemiology, in a country such as Korea where large quantities of Chinese cabbage are consumed, there is no substantiated evidence of the association between the occurrence of goiter and Chinese cabbage consumption. The only association of goiter and *Brassica* consumption has been observed in areas where benign goiter is endemic because of low iodine in the area. Heavy consumption of some *Brassica* crops has caused metabolic disorder in cattle, some breeds of sheep, poultry and so on.

C. G. KUO: Do you think the high levels of glucosinolates in *Brassica* vegetables which are consumed by east Asians in relatively high quantities may promote goiter cases?

P. H. WILLIAMS: If goiter is a problem in an area of high *Brassica* vegetable consumption then glucosinolates intake should be examined as a possible contributing factor.

C. G. KUO: What happens to glucosinolates after cooking or processing, such as kimchi making?

P. H. WILLIAMS: Cooking does not destroy glucosinolates; it can prevent the enzymatic conversion of glucosinolates to goitrins but not necessarily. A study has been made by us on sauerkraut and the fate of glucosinolates in common cabbage. I suspect that in kimchi preparation the same kind of chemical conversion occurs, but in Chinese cabbage different glucosinolates are involved than in common cabbage.

J. WEICHMANN: What was the internal standard used for quantitative GLC? Are the methods for extraction and analysis of glucosinolates published?

P. H. WILLIAMS: A number of internal standards are used depending upon the GLC column materials. Methyl palmitate is used with EGSS-X on Gaschrom Q and Apiezon L on Gaschrom Q. Complete methods for extraction and GLC analysis have been published. Some are given under Literature Cited in this paper. Others by Van Etten *et al* and Daxenbichler *et al* are published mainly in the Journal of Agriculture and Food Chemistry.

J. WEICHMANN: Do you have any idea about the relation between any single substance and the taste?

P. H. WILLIAMS: McLeod at the University of Reading in England has done some work on the individual flavor components of glucosinolates but a great deal more needs to be done.

S. Y. ZEE: Do you know what causes the bitterness in some Chinese cabbage cultivars? Could this bitterness be related to glucosinolates?

P. H. WILLIAMS: Allyl isothiocynate and 3-methylthiopropyl glucosinolates have been associated with bitterness in crucifers. Possibly one or both of these build up during Chinese cabbage growth in summer. Generally younger and faster growing tissues have higher quantities of glucosinolates and these compounds diminish in older or longer growing cultivars.