Distribution of Glucosinolates in the Pith, Cambial-Cortex, and Leaves of the Head in Cabbage, *Brassica oleracea* L.

The distribution of glucosinolates in the pith, cambial-cortex, and leaves were determined for cabbage heads from 13 varieties of white, red, and Savoy types. The cambial-cortex contains about twice the weight concentration of total glucosinolate compared to that in the pith or the leaves. This increased glucosinolate concentration is due primarily to increased amounts of allyl, 3-butenyl, and phenylethyl glucosinolates. The concentrations of 3-methylthiopropyl and 4-methylthiobutyl glucosinolate in the pith were high. In contrast, the amounts of their oxidized counterparts, 3-methylsulfinylpropyl and 4-methylsulfinylbutyl glucosinolates, were high in cambial-cortex and leaves. Of all glucosinolates in the leaves, 3-indolylmethyl glucosinolates comprised 45, 28, and 68% in the white, red, and Savoy types, respectively.

There is some concern that new varieties of cabbage and related vegetables may contain larger amounts of natural toxicants than the varieties from which they are developed (Senti and Rizek, 1974). Glucosinolates are the source of toxicants that may be harmful if consumed in large amounts (VanEtten and Wolff, 1973). To evaluate new introductions, the composition of established varieties must be known. Composition of the leafy portion of 22 varieties of cabbage has been reported (VanEtten et al., 1976), but no analyses of the different morphological parts of the cabbage head are available. Composition of these parts is important because the heart (pith, cambial, and cortical tissues) is discarded in some methods of food preparation, but not in others. The pith is a relatively inactive tissue, whereas the cambial and cortical tissues, referred to in this paper as the cambial-cortex, consists of actively dividing cells from which the leaves of the cabbage head originate. The results presented here show pronounced differences in glucosinolate pattern and concentration among the pith, cambial-cortex, and leaves of cabbage.

EXPERIMENTAL SECTION

Types and varieties of cabbages selected are given in Table I. The varieties were grown in 1976 in Wisconsin, except for Round Up, which was grown in Oregon and in New York. Two representative heads were selected from each of 15 accessions. Three samples were taken from each head; one as a wedge from the leafy part, the second from the cambial-cortex, and the third from the pith. The cambial-cortex, which lies between the pith and the leaf petioles, was separated from those parts by careful trimming with a paring knife. A weighed portion of each part of the head was extracted with boiling methanol. The extract from each sample was kept frozen until ready for analysis. Glucose liberated enzymatically from the glucosinolates was used to calculate total glucosinolates (VanEtten and Daxenbichler, 1977). Specific glucosinolates were measured by gas-liquid chromatography as their aglucon hydrolytic products (Daxenbichler and VanEtten, 1977). 3-Indolylmethyl glucosinolate (3-indolylmethyl-GS) and 3-(*N*-methoxy)indolylmethyl-GS were determined together by measuring thiocyanate ion released by myrosinase added to the extract by a method similar to that of Josefsson (1968).

RESULTS AND DISCUSSION

Composition of Cabbage Types. The glucosinolates of cabbage heads are reported as aglucon products (isothiocyanates, goitrin, thiocyanate ion) in Table II because of the conditions used in the assay procedure (Daxenbichler and VanEtten, 1977). Nitriles, however, may be formed instead if the conditions are proper (Daxenbichler et al., 1977). White and Savoy cabbage leaves and cambial-cortex tend to be high in allyl isothiocyanate (allyl-NCS) and 3-methylsulfinylpropyl-NCS. The red type tends to be relatively high in butenyl-NCS, 4-methylsulfinylbutyl-NCS, and in goitrin, which is derived from 2-hydroxy-3-butenyl-GS. Savoy cabbage is highest in SCN ion, formed from the 3-indolylmethyl-GS's. Savoy and red types tend to be higher in total glucosinolates than the white cabbage. Similar patterns were previously found for the leaves from all three types (VanEtten et al., 1976). In that report the variability between heads within a variety were also given.

Table I. Types and Varieties of Cabbage Analyzed

Savoy, *Brassica oleracea* var. *sabauda* (L.)	
Savoy Ace, hybrid	Savoy Dark Green, open pollinated
White, *B. oleracea* var. *capitata* L. f. *alba* DC.	
Experimental hybrid 1561	Round Up, hybrid grown in New York
Market Prize, hybrid	Round Up, hybrid grown in Oregon
Superette, hybrid	
Red, *B. oleracea* var. *capitata* f. *rubra* (L.) Thell.	
Experimental hybrid 7234, Red	Mammoth Red Rock, open pollinated
Red Acre, open pollinated	Red Head, hybrid
Inbred 4, Red	Red Danish, open pollinated
Inbred 78, Red	

Composition of Parts of Cabbage Head. In preliminary work the top, center, and bottom portions of the cambial-cortex were analyzed separately to see if there was a difference in glucosinolate content in relation to location (where the sample was taken). The bottom section of the cambial-cortex was slightly, but not significantly, lower in total glucosinolate. The pattern as to relative amounts of each kind of glucosinolate was similar. Glucosinolate content was quite low in the older, outer leaves that were not a part of the cabbage head. None of these results are reported in detail.

The cambial-cortex layer contains about twice the concentration of total glucosinolates compared to the pith or the leaves (Table II). Allyl-GS, 4-butenyl-GS, phenylethyl-GS and, to some extent, 3-methylsulfinylpropyl-GS are the major contributors to this increase in glucosinolate concentration in the cambial-cortex. The pith is relatively low in the parent glucosinolates that are the source of flavors such as volatile isothiocyanates and nitriles, as well as thiocyanate ion.

In the pith of the white and Savoy cabbage, 3-methylthiopropyl-GS is found in high concentration, but the more oxidized component, 3-methylsulfinylpropyl-GS, and the unsaturated allyl-GS are high in the cambial-

Table II. Distribution of Aglucons and Thiocyanate Ion in Three Types of Cabbage[a]

aglucon product from glucosinolate	part analyzed	white, ppm	red, ppm	Savoy, ppm
allyl-NCS	pith	42.0	14.0	27.0
	cambial-cortex	141.0	45.0	146.0
	leaf	23.0	8.0	12.0
	LSR[b]	1.3	1.3	1.6
3-methylthiopropyl-NCS	pith	17.0	11.0	68.0
	cambial-cortex	12.0	5.0	34.0
	leaf	3.0	4.4	1.1
	LSR	3.6	2.8	7.7
3-methylsulfinylpropyl-NCS	pith	4.0	6.0	52.0
	cambial-cortex	38.0	28.0	175.0
	leaf	38.0	17.0	85.0
	LSR	4.9	3.5	12.4
3-butenyl-NCS	pith	4.0	15.0	0.0
	cambial-cortex	36.0	105.0	0.0
	leaf	2.0	10.0	2.0
	LSR	1.9	1.7	2.9
5-vinyloxazolidine-2-thione (goitrin)	pith	6.5	19.0	2.9
	cambial-cortex	6.9	18.0	7.8
	leaf	1.8	5.4	0.0
	LSR	2.6	2.2	4.6
4-methylthiobutyl-NCS	pith	13.0	81.0	9.0
	cambial-cortex	1.0	18.0	2.4
	leaf	0.5	2.7	3.0
	LSR	2.2	1.9	3.5
4-methylsulfinylbutyl-NCS	pith	0.6	36.0	0.0
	cambial-cortex	2.0	59.0	0.0
	leaf	4.0	63.0	3.0
	LSR	2.9	2.3	5.4
4-methylsulfonylbutyl-NCS	pith	0.0	1.3	0.0
	cambial-cortex	3.1	7.4	0.0
	leaf	2.2	6.8	5.8
	LSR	4.5	3.3	10.9
benzyl-NCS	pith	0.0	0.0	0.0
	cambial-cortex	1.4	1.2	3.4
	leaf	0.4	0.4	1.3
	LSR	1.8	1.6	2.5
phenylethyl-NCS	pith	0.3	0.5	3.5
	cambial-cortex	33.0	15.0	22.0
	leaf	1.9	1.6	1.6
	LSR	3.7	2.8	7.9
SCN ion, from 3-indolylmethyl-GS's	pith	4.0	6.0	14.0
	cambial-cortex	25.0	47.0	40.0
	leaf	34.0	36.0	122.0
	LSR	2.3	1.9	3.7
total glucosinolates[c]	pith	566.0	819.0	731.0
	cambial-cortex	1494.0	1764.0	2178.0
	leaf	587.0	1014.0	1422.0
	LSR	1.2	1.2	1.4

[a] Ppm in fresh material as received. NCS = isothiocyanate. [b] Ratio of values between parts exceeding the least significant ratio (LSR) indicate statistically significant differences (≅ 0.05 level). The percent relative standard deviation for an aglucon from portions of the same head is approximately RSD = 100 ($LSR^{1.09} - 1$), where LSR is for white varieties. [c] Total glucosinolate calculated from glucose released by myrosinase using the average M_r 457 of the potassium salt of ally, 3-methylsulfinylpropyl, 4-methylsulfinylbutyl, and 3-methylindolyl glucosinolate.

Table III. Concentration of Glucosinolate within Cabbage Head

cabbage type	glucosinolate	head component: pith	cambial-cortex	leaf
white	3-indolylmethyl-GS's, μmol/g	0.06	0.43	0.58
	other individual GS's, μmol/g	0.74	2.39	0.54
	total GS,[a,b] μmol/g	1.24	3.28	1.28
	3-indolylmethyl-GS's, % of total	5.00	13.00	45.00
red	3-indolylmethyl-GS's, μmol/g	0.11	0.80	0.62
	other individual GS's, μmol/g	1.24	2.30	0.76
	total GS,[a,b] μmol/g	1.79	3.85	2.22
	3-indolylmethyl-GS's, % of total	6.00	21.00	28.00
Savoy	3-indolylmethyl-GS's, μmol/g	0.24	0.69	2.10
	other individual GS's, μmol/g	1.14	3.03	0.72
	total GS,[a,b] μmol/g	1.60	4.77	3.11
	3-indolylmethyl-GS's, % of total	15.00	14.00	68.00

[a] From glucose release: see footnote c to Table II. [b] Sum of individual glucosinolates ranges from 62-91% of the total glucosinolates calculated from glucose release.

cortex and leaves. Underhill et al. (1973) reviewed evidence for the interconversions of glucosinolates possessing the same carbon chain. Like white and Savoy cabbage, the more oxidized components of red cabbage are relatively low in the pith. Thus in red cabbage, conversion of 4-methylthiobutyl-GS to the oxidized forms (4-methylsulfinyl- and 4-methylsulfonylbutyl-GS) or to the unsaturated form (3-butenyl-GS) does not occur in the pith to the extent it does in cambial-cortex and leaf.

Pith averaged 2.4% of the head weight with extremes from 1.4–4.2%, cambial-cortex 3.6% (2.7–5.2%), and leaves 94.0% (90.7–95.6%), respectively. Although higher in glucosinolate content, the cambial-cortex probably cannot make a major contribution to human comsumption of glucosinolates or derived products.

Thiocyanate Ion as a Measure of 3-Indolylmethyl-GS's. All 3-indolylmethyl-GS's and *p*-hydroxybenzyl-GS hydrolyze to yield unstable isothiocyanates that decompose to give thiocyanate ion as one of the products. Of these glucosinolates, the only ones identified in cabbage are 3-indolylmethyl-GS and 3-(*N*-methoxy)indolylmethyl-GS, although 3-(*N*-sulfo)indolylmethyl-GS might be present (Elliot and Stowe, 1971). Accordingly, a measure of the thiocyanate ion formed following enzymatic hydrolysis is an indirect measure of the 3-indolylmethyl-GS's. These form other products of biochemical interest: 3-hydroxyindoles, which may react with ascorbic acid to form ascorbigen that is active as vitamin C; 3-indolylacetonitrile, which is a plant hormone formed under certain conditions (Gmelin and Virtanen, 1961).

The values in Table II give the concentrations in parts per million of various products of interest to the food technologist. The 3-indolylmethyl-GS, however, has a molecular weight 8.4 times that of thiocyanate ion, whereas the remaining glucosinolates have molecular weights of only 2.7–4.0 times that of the product analyzed. To provide a better comparison of 3-indolylmethyl-GS with the other glucosinolates in cabbage, the values in Table III are reported as micromoles glucosinolate per gram and as percent of total glucosinolates. Up to 68% of the

glucosinolates in the leafy portion are 3-indolylmethyl-GS's. Because the leafy part averages 94% of the entire cabbage head, the 3-indolylmethyl-GS's make the major contribution of glucosinolates in cabbage.

Certain insects such as *Pieris rapae* and *Hylemya brassicae* (Bouche) display a parasite–host relationship with *Brassica* (Thorsteinson, 1960). The glucosinolates and/or their hydrolytic products are involved in attracting the adult female and in her oviposition. These compounds also act as feeding stimulants for the larvae (Nair and McEwen, 1976; Blau et al., 1978). A knowledge of the variation in the amount and the kind of glucosinolates with respect to the parts of the cabbage plant may require a reevaluation of the relationship of host to parasite.

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25-Hydroxycholecalciferol in Cow Milk as Determined by High-Performance Liquid Chromatography

A high-performance liquid chromatographic (LC) procedure was developed for the determination of 25-hydroxycholecalciferol (25-OH-D_3) in cow milk. The procedure involved extraction with an ethanol–ether mixture, a set of solvent partitions, adsorption chromatography on silica gel, a partition chromatography on diatomaceous earth support, and final determination by reversed-phase LC on a C_{18} bonded microparticulate silica column using a 254-nm fixed wavelength detector. The method was quantitative at the 10-ppb level and capable of detection at the 2–3-ppb level. The method was applicable to milk and colostrum. The endogenous level of 25-OH-D_3 in the milk was below the detection limit. It was very significant that, even in cows whose serum concentrations were elevated fivefold after treatment with 25-OH-D_3, the concentration in the milk was below the detection level.

The metabolite of cholecalciferol (vitamin D_3), 25-hydroxycholecalciferol (25-OH-D_3) was found useful in reducing the incidence of parturient paresis in cows (Bringe et al., 1971; Olson et al., 1973). We have reported procedures for the determination of 25-OH-D_3 in cow plasma (Koshy and VanDerSlik, 1976, 1978) and in cow liver, kidney, and muscle (Koshy and VanDerSlik, 1977). Since it was known to us that the serum level of 25-OH-D_3 was elevated after oral, intramuscular, or intravenous administration of 25-OH-D_3, it was of great importance to know if there was any corresponding increase in the concentration of 25-OH-D_3 in the milk. The present study was undertaken to determine the concentration of 25-OH-D_3 in the milk and colostrum of treated and untreated dairy cattle.

EXPERIMENTAL SECTION

Sample. A 100-g sample was used for each analysis. If the sample was to be stored for any length of time, it was weighed into individual plastic containers and frozen.

Solvents. All solvents except 3A alcohol and anhydrous ethyl ether were distilled in glass (Burdick and Jackson, Inc., Muskegon, MI).

Extraction. The thawed sample was transferred to a

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