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Glucosinolate Products in Commercial Sauerkraut

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Three varieties of cabbage, Roundup, Sanibel, and TBR Globe, were commercially processed into sauerkraut. The aglucon products from two of the three glucosinolates abundant in kraut cabbage were measured at intervals from fresh cabbage to canning of the sauerkraut. Within the first 2 weeks of fermentation, all the glucosinolates were hydrolyzed. Thiocyanate ion and 1-cyano-3-methylsulfinylpropane, the aglucon products measured, varied little from 2 weeks of fermentation to finished sauerkraut. In the finished kraut, thiocyanate ion ranged from 9 to 17 ppm and 1-cyano-3-methylsulfinylpropane ranged from 16 to 25 ppm. No isothiocyanates or goitrin were present. No nitriles from allyl glucosinolate were found.

A number of potential toxicants occur in cruciferous vegetables, but apparently pose no human health hazard as currently used. To evaluate whether new varieties with possible increased toxicant levels might pose a health problem (Senti and Rizek, 1974), it is necessary to know both the composition of current varieties and the effects of food processing on the toxicants. The toxic properties of glucosinolates that are found in cruciferous vegetables depend in part on the type of aglucon products formed from them during food processing.

Glucosinolates may hydrolyze to form nitriles instead of isothiocyanates in a number of cruciferous crops, depending upon the treatment of the plant material and the conditions during hydrolysis (VanEtten et al., 1966; VanEtten and Daxenbichler, 1971; Tookey, 1973). Cabbage leaves autolyze to form organic nitriles from the glucosinolate aglucons (Daxenbichler et al., 1977). The present study was undertaken because preliminary evidence indicated that nitriles from glucosinolates were found in commercial sauerkraut preparations (Daxenbichler et al., 1977).

EXPERIMENTAL SECTION

Fresh Cabbage Sample Preparation. Three varieties of cabbage, Roundup grown in Oregon and in New York and Sanibel and TBR Globe grown in Wisconsin, were each commercially harvested and processed into sauerkraut in 1976. Five representative heads of each cabbage variety and location were selected. Hot methanol extracts were prepared and analyzed as described for glucosinolate aglucons (Daxenbichler and VanEtten, 1977) and total glucosinolate (VanEtten and Daxenbichler, 1977). These methods are outlined in Daxenbichler et al. (1979).

Fermented Cabbage Sample Preparation. Samples were collected at weekly intervals during the sauerkraut fermentation and were held frozen at −18 °C until just before analysis. The final sauerkraut sample, a commercially canned product, was held under refrigeration until analysis. These fermentation samples were separated into juice and residue by draining through cheesecloth. The juice was analyzed separately. Juice and residue were also recombined in the same proportion as the original to obtain samples of the whole fermentation. The recombined samples were treated with hot methanol as described by Daxenbichler et al. (1979). The juice was analyzed without hot methanol treatment.

Salt and Lactic Acid. These substances were determined by the method of VanEtten (1978).

Thiocyanate Ion. Assay for thiocyanate ion in fresh cabbage was made by a method similar to that of Josefsson (1968). In this method the optical absorbance of the ferric–thiocyanate complex is measured both before and after decomposition of the complex with mercuric ion. The absorbance after decomposition provides a correction for background interferences. Addition of thiocyanate ion to fermentation samples containing salt and lactic acid demonstrated that only about 16% of the ferric–thiocyanate complex was decomposed by mercuric ion. To overcome this problem, thiocyanate ion was estimated by measuring the colored complex and subtracting the sample solution (without ferric nitrate) as a blank. Under these conditions, quantitative recovery of added thiocyanate ion was then obtained.

1-Cyano-3-methylsulfinylpropane. The 1-cyano-3-methylsulfinylpropane is relatively nonvolatile and elutes where there is little or no interference from other compounds on both EGSSX and Apiezon columns described by Daxenbichler and VanEtten (1977). However, gas–liquid chromatography (GLC) estimation of some aglucon nitriles in the fermentation samples was not possible because of interfering peaks from unknown substances in the dichloromethane extracts.

Satisfactory conditions for complete extraction of 1-cyano-3-methylsulfinylpropane from the fermentation samples were found empirically. Known amounts of pure 1-cyano-3-methylsulfinylpropane added were recovered by the following procedure: A sample of 25 mL of aqueous extract or juice (pH 3.5 to 4.0) was adjusted to pH 8.0–8.8

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Table I. Glucosinolate Content[a] of Cabbages Used for Sauerkraut Fermentation

	Roundup (NY)		Roundup (OR)		Sanibel (WI)		TBR Globe (WI)	
glucosinolate	mean	range	mean	range	mean	range	mean	range
allyl-GS[b]	152	60-289	144	120-172	148	112-209	209	172-221
3-methylthiopropyl-GS	9.1	2.7-12	27	15-39	45	15-76	12	2-33
3-methylsulfinylpropyl-GS	142	65-190	122	108-133	133	79-198	144	74-260
3-butenyl-GS	7.2	0-18	7.2	3.6-11	11	7.2-14	11	2.5-18
2-hydroxy-3-butenyl-GS	10	3.3-37	3.3	0-6.7	27	23-33	10	0-13
4-methylthiobutyl-GS	0.3	0-1.1	2.5	0-8.4	14	2.8-22	0.6	0-0.6
4-methylsulfinylbutyl-GS	11	0-21	2.4	0-5.3	19	5.3-29	5.3	0-26
4-methylsulfonylbutyl-GS	5.0	0-10	0		5.0	0-20	0	
benzyl-GS	0.6	0-1.5	0.3	0-1.2	1.5	1.1-1.5	0.6	0-2.1
2-phenylethyl-GS	6.9	0-11.3	11.3	5.7-25	2.8	2.0-5.7	8.5	5.7-21
3-indolylmethyl-GS[c]	143	42-185	101	76-118	50	25-76	126	76-244
total glucosinolate[d,e]	680	317-1105	509	475-546	608	462-790	654	488-894
head wt, kg	3.2	2.8-4.5	3.6	2.6-4.2	3.9	2.8-6.5	2.0	1.8-20

[a] Potassium glucosinolate values reported in ppm of fresh weight; head weight reported in kilograms. [b] GS = glucosinolate. [c] Also includes 3-(*N*-methoxyindolyl)methyl-GS. [d] Total glucosinolate from glucose released by thioglucosidase (myrosinase) hydrolysis calculated from the average molecular weight (457) of the major glucosinolates as potassium salts. [e] Sum of individual glucosinolates ranges from 72 to 83% of the total glucosinolates calculated from glucose released.

Table II. Glucosinolate Hydrolysis Products in Fermentation[a]

	SCN ion, ppm		1-cyano-3-methylsulfinylpropane, ppm	
sample	mean	range	mean	range
Roundup from New York				
fresh cabbage[b]	17.0 (5)	5-22	40 (5)	18-54
fermentation, 2 wk	21.2 (4)	20-23	37.6 (2)	34-42
fermentation, 3 wk	21.5 (4)	21-22	47.4 (2)	46-69
just before canning, 19 wk	23.2 (6)	18-28	51.6 (2)	51-52
canned product	17.3 (7)	11-26	24.9 (4)	23-27
Roundup from Oregon				
fresh cabbage[b]	12 (5)	9-14	34 (5)	30-38
just before canning, 28 wk	15.1 (4)	14-17	24.8 (2)	23-26
canned product	11.6 (5)	8-16	17.4 (4)	17-20
Sanibel from Wisconsin				
fresh cabbage[b]	6 (5)	3-9	38 (5)	22-56
fermentation, 1 wk	11.5 (4)	11-12	30.0 (2)	28-32
fermentation, 2 wk	13.6 (4)	13-14	27.2 (2)	26-28
just before canning, 17 wk	6.1 (4)	5.9-6.4	15.9 (3)	14-18
canned product no. 1	8.7 (4)	7.8-9.5	15.8 (2)	15-16
canned product no. 2	10.7 (4)	10-12	20.0 (2)	20-20
TBR Globe from Wisconsin				
fresh cabbage[b]	15 (5)	9-29	41 (5)	21-74
fermentation, 2 wk	17.9 (4)	14-22	23.0 (2)	23-23
just before canning, 7 wk	16.4 (4)	14-18	25.6 (3)	23-27
canned product no. 1	10.3 (6)	10-12	24.3 (3)	26-29
canned product no. 2	9.5 (1)		19.0 (2)	18-20

[a] The standard deviation based on variation between determinations on the same sample was 1.49 (27 df) for SCN ion and 1.77 (8 df) for nitrile. On the basis of a single determination, there is 1 chance in 20 that the SCN ion is in error more than 2.9 and the nitrile more than 4.1. Number of assays in parentheses. [b] These values represent the maximums if all the 3-indolylmethyl or 3-methylsulfinylpropyl glucosinolate in cabbage was converted to SCN ion or 1-cyano-3-methylsulfinylpropane, respectively.

with dilute sodium hydroxide and saturated with sodium chloride. Following centrifugation, the supernatant solution was extracted with 100 mL of pentane–hexane to remove lipid-like materials. The aqueous phase was extracted with 3 × 100 mL of dichloromethane, and the combined dichloromethane extracts were dried with anhydrous sodium sulfate. The filtered extract was evaporated to ca. 3 mL and transferred to a 5–10-mL vial. Excess dichloromethane was evaporated with a stream of nitrogen, and methyl palmitate internal standard was added for the GLC assay. The elution positions and correction factor for 1-cyano-3-methylsulfinylpropane were determined with pure compound prepared from parent glucosinolate (glucoiberin) as described by Daxenbichler et al. (1977). Identity of the nitrile was confirmed by tandem gas chromatography–mass spectroscopy (Spencer and Daxenbichler, 1980).

1-Cyano-2,3-epithiopropane. Fermented cabbage (200 g) was blended with 800 mL of pentane–hexane and drained through cheesecloth. The juice was then extracted three more times with pentane–hexane (800 mL). The combined pentane–hexane extracts were carefully concentrated below 20 °C in a rotary evaporator to a volume of 200 mL. A 50-mL aliquot was further concentrated to a volume of about 3 mL and applied to a silica cartridge, Sep-Pak, Waters Associates. The cartridge was flushed with 5–10 mL of pentane–hexane, followed by 4 mL of hexane–dichloromethane (1:1, v/v). Material for GLC analysis was eluted from the cartridge with 4 mL of dichloromethane. The eluate was collected in a vial kept immersed in ice water and was concentrated to about 0.3 mL with a stream of nitrogen. Authentic 1-cyano-2,3-epithiopropane (from *Brassica juncea* seed) was added to sauerkraut to test the validity and sensitivity of the method.

Other Analytical Methods. Isothiocyanates were converted to thioureas (Appelqvist and Josefsson, 1967). These and goitrin were measured at 244 nm (UV) in the dichloromethane extracts prepared as described for 1-cyano-3-methylsulfinylpropane. Intact glucosinolates were measured by a modification of the method of VanEtten and Daxenbichler (1977). The amount of ion-exchange resin was doubled (6 mL) to give quantitative adsorption of the glucosinolates in the presence of lactic acid and sodium chloride.

RESULTS AND DISCUSSION

Glucosinolate Content of Cabbage. Composition of the four cabbage samples used for fermentation is given in Table I in terms of individual glucosinolates and total glucosinolates. All accessions are high in allyl and 3-methylsulfinylpropyl glucosinolates that form the corresponding isothiocyanates or nitriles. The cabbages are also high in indolylmethyl glucosinolates that, when hydrolyzed, undergo further changes to release SCN ion (Gmelin and Virtanen, 1961). Such a glucosinolate pattern is typical for white cabbages (VanEtten et al., 1976).

Lactic Acid and Sodium Chloride. After the first 2 weeks of fermentation, lactic acid ranged from 0.6 to 2.3% and salt content from 1.3 to 3.1%. No further changes were noted during the remainder of the 6–28-week fermentation periods. Variation of lactic acid and salt appeared to be related to lack of uniformity within the vats. Lactic acid and salt in the finished kraut were lower and more uniform: 0.8–1.2% lactic acid and 1.0–2.0% salt. The results for the finished kraut are similar to those previously reported by Pederson and Albury (1969). The values for the whole product are usually lower than those for the juice alone.

Fate of Glucosinolates during the Fermentation. The cabbage was analyzed for unhydrolyzed glucosinolates before and after 1 and 2 weeks of fermentation. In no case were any unhydrolyzed glucosinolates found after 2 weeks. Tests for goitrin and for isothiocyanates were negative throughout the fermentation.

Thiocyanate ion was present at a higher level than that expected from the indolylmethyl glucosinolates measured in fresh cabbage (Table II). This probably is due to the modification of the method for thiocyanate ion required for the fermentation samples as well as lack of homogeneity within the vats.

During fermentation, 1-cyano-3-methylsulfinylpropane is somewhat variable but represents half of that theoretically possible. The 1-cyano-3-methylsulfinylpropane present in the processed sauerkraut from Roundup (New York), Roundup (Oregon), Sanibel (Wisconsin), and TBR Globe (Wisconsin) varieties represents 62, 51, 47, and 53%, respectively, of that which could be obtained from the parent glucosinolates present in the fresh cabbage (Table II). Losses that occurred at canning may be due to heat applied at this step and to the drainage of the juice, which contained larger amounts of water solubles than did the drained solids.

Allyl glucosinolate, the third major glucosinolate present in the fresh cabbage, may be hydrolyzed to allyl isothiocyanate, allyl cyanide, or 1-cyano-2,3-epithiopropane. Allyl isothiocyanate in sauerkraut was excluded by the negative test for isothiocyanates. One might expect that this glucosinolate, under the conditions of shredding, would be hydrolyzed to a mixture of 1-cyano-2,3-epithiopropane and allyl cyanide. The latter is too volatile to be measured by the methods used. Juice from both early fermentation samples and finished sauerkraut showed no 1-cyano-2,3-epithiopropane although the procedure can easily detect 1 ppm. Two additional sauerkrauts purchased from local grocers also failed to show any of this nitrile. Thus, the fate of allyl glucosinolate in sauerkraut production remains unclear.

From a practical viewpoint, the most important observation reported here is that nonvolatile 1-cyano-3-methylsulfinylpropane is found equal to about half the maximum amount that could be formed from its precursor glucosinolate. The presence of this nitrile suggests that it should be tested for possible chronic toxicity at low levels of consumption.

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