tion even when sodium sulfate was included during extraction (data not included). Water was an efficient extractant of hexazinone from the plant material. However, certain pigments were extracted with all solvents more polar than methanol, and aqueous solutions of nonpolar solvents that could not be easily removed by the cleanup process utilized in this procedure. It was determined that aqueous methanol solutions higher than 10% resulted in interfering peaks on the chromatogram, and less than 10% aqueous methanol did not improve extraction efficiency for hexazinone (data not included).

Generally, the total extraction procedure, utilizing methanol, consistently accounted for 81 ± 2% of the hexazinone added to the alfalfa foliage samples. Analysis of data from hexazinone lost at each step in the procedure indicated that much of this loss is during stages subsequent to the initial extraction stage (Table II) and this loss is adjusted when using the IS to quantify hexazinone in plant tissue samples (Table I). Also, use of an IS adjusts for variations in extraction efficiency and instrument sensitivity.

This procedure of quantifying hexazinone was determined to be simple, inexpensive, accurate, and reproducible. Also, the sensitivity range of this method is adequate for most research with hexazinone in plant tissue. The choice of extractants will depend on pigments in the plant material to be extracted. Methanol was effective for alfalfa tissue; however, other species may contain interfering pigments that are extracted with methanol, and methanolic solutions of nonpolar solvents could be more effective extractants.

Registry No. Hexazinone, 51235-04-2.

LITERATURE CITED

Baron, J. J.; Monaco, T. J. Uptake, Translocation, and Metabolism of Hexazinone in Blueberry (*Vaccinium* sp.) and Hollow Goldenrod (*Solidago fistulosa*). *Weed Sci.* **1986**, *34*, 824.

Hatzios, K. K.; Howe, C. M. Influence of the Herbicides Hexazinone and Chlorsulfuron on the Metabolism of Isolated Soybean Leaf Cells. *Pestic. Biochem. Physiol.* **1982**, *17*, 207.

Holt, R. F. Determination of Hexazinone and Metabolite Residues Using Nitrogen-Selective Gas Chromatography. *J. Agric. Food Chem.* **1981**, *29*, 165.

McIntosh, C. L.; Schlueter, D. D.; Holt, R. F. Hexazinone. *Anal. Methods Pestic. Plant Growth Regul.* **1984**, *13*, 267.

McNeil, W. K.; Stritzke, J. F.; Basler, E. Absorption, Translocation, and Degradation of Tebuthiuron and Hexazinone in Woody Species. *Weed Sci.* **1984**, *32*, 739.

Pease, H. L.; Holt, R. F. Improved Method for Determining Benomyl Residues. *J. Assoc. Off. Anal. Chem.* **1971**, *54*, 1399.

Weed Science Society of America. Hexazinone. In *Herbicide Handbook of the Weed Science Society of America*; Beste, C. E., Ed.; Weed Science Society of America: Champaign, IL, 1983.

Received for review March 7, 1988. Accepted July 29, 1988. This research was supported by State and Hatch funds allocated to the Georgia Agricultural Experiment Stations. The assistance of Donna Wyatt is gratefully acknowledged.

Rapid Acid Hydrolysis of Plant Cell Wall Polysaccharides and Simplified Quantitative Determination of Their Neutral Monosaccharides by Gas–Liquid Chromatography

Christine Hoebler,* Jean Luc Barry, Agnès David, and Jean Delort-Laval

A rapid method for the determination of plant cell wall neutral polysaccharides is described. A two-step acid hydrolysis procedure, suitable for a wide range of plant cell wall materials, has been developed. The effect of several parameters (substrate particle size, reaction time, temperature) on the hydrolysis rate of various substrates (microcrystalline cellulose, wheat straw, beet pulp, soybean hull, sunflower husk) has been studied. Among tested parameters, sample particle size and primary hydrolysis temperature predominantly affect acid degradation of plant cell wall polysaccharides. Maximal substrate hydrolysis rate is obtained with finely ground materials (average particle size 80×10^{-3} mm) submitted to a 30-min primary hydrolysis carried out at 25 °C in 72% sulfuric acid, followed by a 120-min secondary hydrolysis in boiling 2 N sulfuric acid. Neutral sugars released by hydrolysis are quantitatively determined, after reduction and acetylation, by gas–liquid chromatography. In the optimized procedure, alditol acetates are totally recovered and reagents interfering in chromatographic separation are eliminated. The validity of the proposed procedure has been tested with various plant materials.

Several sophisticated analytical methods for quantitative determination of cell wall polysaccharides and their neutral sugars have been proposed. They usually involve sugars release by acid hydrolysis and their separation and determination by gas–liquid chromatography (Dutton, 1973). Current procedures for preparing volatile derivates of sugars are not suitable for routine analysis in nutrition investigation. They have therefore not been frequently applied to digestion studies of food polysaccharides (Graham et al., 1986; Nyman and Asp, 1982). Rapid analysis of cell wall sugars, applicable to various substrates and to digesta samples, is necessary in nutritional experiments. The present work describes several adaptations, with the aim of obtaining the most accurate method of determination of nonstarch polysaccharides in various substrates. At first, the possibility of defining a standardized hydrolysis procedure using finely ground materials was investigated; in the second part, a method for alditol acetate preparation (Blakeney et al., 1983; Harris et al., 1988) has been shortened and improved: In the modified procedure, alditol acetates are completely extracted and,

Laboratoire de Technologie Appliquée à la Nutrition, Institut National de la Recherche Agronomique, Rue de la Géraudière B.P. 527, 44026 Nantes Cedex 03, France.

0021-8561/89/1437-0360$01.50/0

after adequate purification of the extract, the derived sugars are quantitatively analyzed by gas chromatography.

The hydrolysis method has been designed to cleave glycosidic bonds in lignocellulosic substrates. The hydrolysis conditions influence the recovery of neutral sugars. The released sugars, more or less stable in acid medium, are partially transformed into furfural derivatives (Feather and Harris, 1973; Dutton, 1973).

Cellulose crystallinity and lignin are the main barriers to chemical or enzymatic degradation. In order to alter the fine structure of cellulose or disrupt lignin bonds, pretreatment must be applied to increase cellulose degradation by hydrolytic reagents without modification of noncellulosic polysaccharides. Among physical and chemical procedures (Abou-State et al., 1983; Philip et al., 1979), fine grinding appears most promising to overcome the effects of lignin barrier and of cellulose crystallinity (Millet et al., 1979). It has been shown that particle size reduction and surface area increase by ball milling are probably more important than reduction of crystallinity (Caulfield and Moore, 1974; Thomart et al., 1983). For analytical purposes, several authors have studied rapid and complete hydrolytic sequences, using various acids, e.g. sulfuric acid (Saeman et al., 1954; Selvendran et al., 1979), trifluoroacetic acid (Fengel and Wegener, 1979; Paice et al., 1982; Albersheim et al., 1967), and hydrogen fluoride (Hardt and Lamport, 1982). None of these procedures are suitable for all types of cell wall substrates and for routine analysis; the Saeman procedure (Saeman et al., 1954) for total hydrolysis of wood samples is the one most widely used. Under its hydrolysis conditions, decomposition of the most degradable free monosaccharides (pentoses) cannot be avoided (saeman et al., 1954; Dutton, 1973). An alternative milder two-step hydrolysis, suitable for lignocellulosic residues, in which varying hydrolysis conditions are applied to plant cell wall materials, is more time consuming.

Neutral sugars released by acid hydrolysis are generally quantified by gas–liquid chromatography as their alditol acetates (Albersheim et al., 1967; Theander and Aman, 1979), aldonitrile acetates (Schweizer and Würsch, 1979), or trimethylsilyl derivates (Mason and Slover, 1971; Ford, 1974) or by high-pressure liquid chromatography (Paice et al., 1982; Slavin and Marlett, 1983).

Acetylated alditols solutions are stable at high temperatures; therefore, they are widely used for quantitative determination of sugars released in hydrolysates of plant cell wall materials (Albersheim et al., 1967; Carré and Brillouet, 1986; Oades, 1967). Aldoses are reduced by sodium borohydride; the removal of borate is not necessary to achieve quick and complete acetylation of alditol, when using 1-methylimidazole, a powerful catalyst first proposed by Connors and Pandit (1978) and later used by several authors (Englyst and Cummings, 1984; Bittner et al., 1980; Blakeney et al., 1983, Harris et al., 1988).

MATERIALS AND METHODS

Reagents and Samples. High-purity monosaccharides and reagents are used for all analysis. Avicel microcrystalline cellulose (FMC Co.) is used as a reference substrate. Other products (wheat straw and bran, sunflower husk, sugar cane bagasse, soybean hull, beet pulp, copra meal, citrus pulp) are of commercial origin. Xylan from oat spelts is purchased from Sigma.

Analytical Methods. The particle size of samples is determined in an ammonium thiocyanate–isopropyl alcohol (4:96, w/v) suspension by Coulter-Counter (Coultronics Model TA2, 16 channels), equipped with measurement cells for maximum particle size of 80×10^{-3} or 280×10^{-3} mm). Plotting the results on a log probability scale allows for graphic determination of particle size mean diameter and geometric standard deviation (Melcion and Delort-Laval, 1979). Moisture content of samples is determined by drying at 100 °C for 8 h. Hemicellulose content is determined by the neutral detergent fiber method, and cellulose and lignin contents are determined by the acid detergent fiber method (Goering and Van Soest, 1970). Total reducing sugars after acid hydrolysis are analyzed by the ferricyanide automated method (Hoffman, 1937).

Sample Preparation. The substrates are ground in a water-cooled laboratory grinder (IKA) so as to pass through a 400×10^{-3} mm sieve. A smaller particle size is obtained by regrinding part of the ground sample previously cooled at −5 °C in a ball mill so as to pass through a 160×10^{-3} mm sieve. In order to avoid the interference of soluble sugars in the chromatography study, a 5-g sample is suspended in 25 mL of 80% ethanol, at room temperature, stirred continuously for 10 min, and then centrifuged. This operation is repeated twice. After being dried at 40 °C under vacuum, samples are reground in a ball mill for 5 min.

Hydrolysis Procedure. For the hydrolysis study, the sample (0.1 g) is mixed in 1.25 mL of 72% (w/w) sulfuric acid with a glass stick. Primary hydrolysis is performed for 0, 15, 30, or 60 min at 25 or 30 °C. The mixture is then diluted with 13.5 mL of water and kept in a boiling water bath for 0, 30, 60, 90, 120, or 240 min; after cooling and addition of 3.1 mL NaOH 32% (w/v), solutions are centrifuged and total reducing sugars are measured in the supernatant.

For gas–liquid chromatography analysis, a 20-mg sample is mixed in 0.25 mL of 72% (w/w) sulfuric acid with a glass stick. Two hydrolysis procedures are used: (i) In the usual method derived from the Saeman et al. (1954) procedure, the primary hydrolysis lasts 120 min at ambient temperature for beet pulp and citrus pulp, 180 min for wheat bran, and 240 min for the other substrates. The secondary hydrolysis is performed according to Selvendran and Du Pont (1980). (ii) In the optimized procedure, primary hydrolysis is performed at 25 °C for 30 min. The acid is diluted with water (1.7 mL) and 1 mL of inositol solution (2 g/L) is added as an internal standard. The mixture is then heated for 120 min at 100 °C. After cooling, the solution is made alkaline by addition of 0.6 mL of 25% (w/w) ammonia solution.

Gas–Liquid Chromatographic Determination of Neutral Sugars. For reducing monosaccharides, 2 mL of sodium borohydride in dimethyl sulfoxide (2:100, w/v) is added to 0.2 mL of neutralized hydrolysate. The mixture is stirred for 90 min at 40 °C; then 0.2 mL of glacial acetic acid is added to decompose excess sodium borohydride. After cooling, acetylation of reduced sugars is performed by adding 4 mL of acetic anhydride and 0.4 mL of 1-methylimidazole to the solution. The mixture is kept for 10 min at room temperature, and then 20 mL of water is added to decompose the excess of acetic anhydride. After cooling, 8 mL of dichloromethane is added and the mixture vigorously shaken for total alditol acetates extraction. The bulk of the upper layer is removed by aspiration, and the lower phase is washed three times with 20 mL of water. The dichloromethane extract is evaporated at 40 °C under vacuum, and alditol acetates are dissolved in 1 mL of dichloromethane and stored at −20 °C. Alditol acetates are separated on a 30 m × 0.25 mm (i.d.) silica capillary column DB 225 (J. & W. Scientific, Inc.) attached to a DELSI 300 chromatograph apparatus fitted with a flame ionization detector and a split injector.

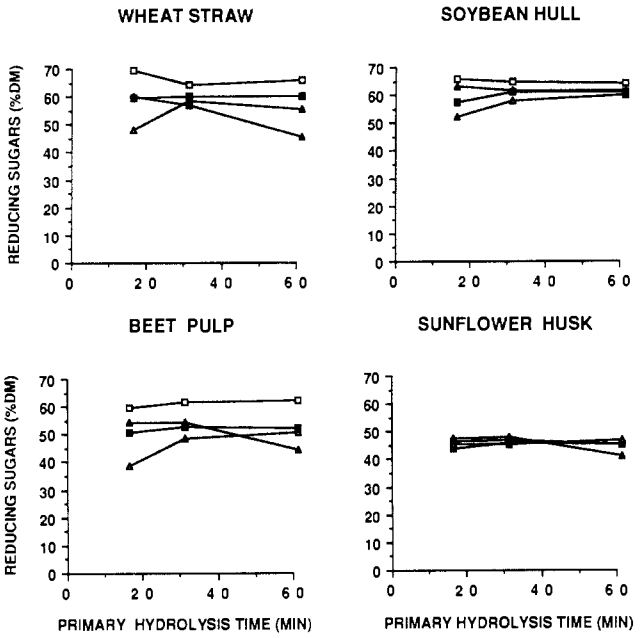


Figure 1. Effect of particle size and prehydrolysis time on reducing sugars yield. The substrates are dissolved in 72% (w/w) sulfuric acid for various times and hydrolyzed in 2 N acid at 100 °C for 240 min. The primary hydrolysis is carried out under the following conditions. Temperature: (□, ■) 25 °C; (△, ▲) 30 °C. Particle size: (□, △) 160 × 10^{-3} mm; (■, ▲) 400 × 10^{-3} mm.

Table I. Particle Size of Microcrystalline Cellulose and Plant Cell Wall Samples

	standard		finely ground	
substrate	mean diam × 10^{-3}, mm	geom std dev	mean diam × 10^{-3}, mm	geom std dev
microcrystalline cellulose			40	1.4
wheat straw	130	2	72	1.5
soybean hull	125	5	86	1.5
sunflower husk	154	1.7	60	1.6
beet pulp	125	2.3	88	1.7

High-purity hydrogen is used as a carrier gas at a flow rate of 1.1 mL min^{-1}. The column temperature is maintained at 220 °C, and the injector port and detector are heated at 270 and 250 °C, respectively, and a 1.5-μL sample in dichloromethane is injected through a glass-lined splitter, set at a 1/90 ratio.

For purposes of comparison, alditol acetates prepared according to Selvendran and Du Pont (1980) are analyzed by gas–liquid chromatography on a glass column (280 × 0.2 cm) of 3% SP 2340 on Supelcoport (100–120 mesh) at 220 °C.

Statistical Analysis. The effects of hydrolysis parameters, sample particle size, temperature, and time of primary hydrolysis and secondary hydrolysis on the yield of reducing sugars and their interaction are evaluated by variance analysis (Snedecor and Cochran, 1979)

RESULTS AND DISCUSSION

Hydrolysis Parameters. (a) Particle Size. The effect of plant material particle size reduction on hydrolysis rate is shown in Figure 1 with primary hydrolysis performed at 25 or 30 °C for 15, 30, or 60 min and secondary hydrolysis carried out for 120 min. Substrate particle sizes are given in Table I.

Sugar liberation from cell wall substrates is significantly different according to particle size and primary hydrolysis temperature (Table II). For all substrates but sunflower,

Table II. Four-Way Variance Analysis for Determining Optimal Hydrolysis Conditions for Various Plant Cell Wall Materials[a]

	deg of freedom	sum of square	mean square
S	3	3260	1087**
PS	1	53	53*
PHT°	1	576	576**
PHT	2	58	29
interaction			
S × PS	3	41	14
S × PHT°	3	336	112**
S × PHT	6	80	13
PS × PHT°	1	357	357**
PS × PHT	2	166	166**
PHT° × PHT	2	46	23
residue	71	771	11
total	95	5744	

[a] Key: S = substrate (wheat straw, beet pulp, sunflower husk, soybean hull); PS = particle size; PHT° = primary hydrolysis temperature (25, 30 °C); PHT = primary hydrolysis time (15, 30, 60 min). Probability: **, <0.01; *, <0.05.

Table III. Plant Cell Wall Composition (% DM) by Triplicate Van Soest Analysis[a]

substrate[a]	cellulose	hemicellulose	lignin
wheat straw	44.89 ± 0.25	31.08 ± 0.11	8.56 ± 0.11
soybean hull	43.51 ± 0.21	19.57 ± 0.33	2.04 ± 0.13
sunflower husk	36.70 ± 0.19	17.26 ± 0.16	21.19 ± 0.09
beet pulp	20.64 ± 0.06	24.48 ± 0.27	2.01 ± 0.05

[a] Particle size 0.5 mm.

the highest hydrolysis rates are obtained (Figure 1) when the primary hydrolysis is performed at 25 °C with finely ground materials (mean particle size 80 × 10^{-3} mm); with coarser samples (mean particle size 160 × 10^{-3} mm), the sugar production varies according to the primary hydrolysis time (Figure 1). Different rates of hydrolysis, according to substrate particle size, explain the significant interactions particle size and hydrolysis temperature (PS × PHT°) and particle size and primary hydrolysis time (PS × PHT) (Table II). Unlike other substrates, hydrolysis rates obtained with sunflower husk are similar whatever the hydrolysis condition (the interaction substrate and primary hydrolysis temperature (S × PHT°) is significant). This result is surprising: A high hydrolysis rate was expected as a result of the fine grinding of this highly lignified material. On the whole, when the particle size of the samples is reduced, it is possible to get an efficient hydrolysis of polysaccharides, with a short time and low temperature for primary hydrolysis.

(b) Hydrolysis Time and Temperature. The composition of the cell wall substrates used in the present study is given in Table III. Primary hydrolysis is performed for 15, 30, or 60 min at either 25 or 30 °C and secondary hydrolysis at 100 °C for 0–240 min.

The primary hydrolysis temperature greatly affects maximum sugar recovery ($P < 0.01$), with all substrates but sunflower husk: Interaction between substrate and primary hydrolysis temperature (S × PHT°) is therefore highly significant (Table IV). When primary hydrolysis is performed at 30 °C, the hydrolysis curves are quite irregular (interaction between primary hydrolysis temperature and secondary hydrolysis time (PHT° × SHT) is significant; Table IV). Reducing sugar content after 90, 120, or 240 min of secondary hydrolysis is significantly ($P < 0.01$) higher when primary hydrolysis is performed at 25 °C rather than 30 °C. For microcrystalline cellulose, mean reducing sugar recoveries are, respectively, 100.9 ± 0.8% and 89.0 ± 1.2% when primary hydrolysis is per-

Table IV. Four-Way Analysis of Variance for Determining Optimal Hydrolysis Parameters for Various Plant Cell Wall Materials

	deg of freedom	sum of square	mean square
S	4	54776	13694**
PHT°	1	2313	2313**
PHT	2	124	62**
SHT	2	17	9
interaction			
S × PHT°	4	993	248**
S × PHT	8	451	56**
S × SHT	8	98	12**
PHT° × PHT	2	65	33**
PHT° × PHT	2	141	71**
PHT × SHT	4	102	26**
residue	142	506	4
total	179	59588	

[a] Key: S = substrate (microcrystalline cellulose, wheat straw, beet pulp, sunflower husk, soybean hull); PHT° = primary hydrolysis temperature (25, 30 °C); PHT = primary hydrolysis time (15, 30, 60 min); SHT = secondary hydrolysis time (90, 120, 240 min). Probability: **, <0.01.

formed at 25 or 30 °C. Several authors have commented upon reactions occurring during hydrolysis. Hardell and Theander (1970) have isolated glucose sulfate esters, produced during the treatment of cotton cellulose samples in acid medium. A higher temperature tends to increase the degree of sulfatation (Turvey, 1965). If the hydrolysis conditions of the secondary step are not strong enough, glucose is lost as sulfate ester; that could explain the apparently lower hydrolysis rate observed with a primary hydrolysis performed at 30 °C.

The primary hydrolysis duration is a significant parameter (Table IV). Its optimum differs according to substrates and primary hydrolysis temperature; this result explains the significant interaction between the substrates and the primary hydrolysis conditions (Table IV). The effect of secondary hydrolysis duration (90, 120, 240 min) is not significant, but for microcrystalline cellulose. With primary hydrolysis performed at 25 °C for 30 min and secondary hydrolysis for at least 120 min, microcrystalline cellulose is totally hydrolyzed (Figure 2). The same hydrolytic conditions are effective for other substrates tested, whose lignin content is lower than 20%. To ensure total hydrolysis of nonstarch polysaccharides of various substrates, samples must be finely ground to a mean particle size of about 80×10^{-3} mm; their primary hydrolysis should be performed for 30 min at 25 °C and their secondary hydrolysis in 2 N H_2SO_4 at 100 °C for at least 120 min.

The sugar loss under mild conditions of primary hydrolysis has been studied. Oat hemicellulosic sugar recovery was the same with or without primary hydrolysis (Table V). Similar results have been obtained by Selvendran et al. (1979) for cell wall fractions of potato tubers. On the other hand, monosaccharides are stable after 120-min heating at 100 °C in 2 N H_2SO_4, as shown by several authors (Selvendran et al., 1979; Montreuil et al., 1965). On the whole, the proposed procedure allows for a maximum free monosaccharide yield from cell walls in lignocellulosic substrates.

Quantitative Determination of Monosaccharides as Alditol Acetates. Under the operating conditions described by Blakeney et al. (1983), only 45% of the dichloromethane used for extraction and 80% of total alditol acetates are recovered. The next assay aimed at defining operating conditions, which would ensure total alditol acetates recovery whatever their concentrations, without increasing DMSO and 1-methylimidazole coextraction.

Table V. Neutral Sugars Released from Oat Xylan by Different Hydrolytic Procedures[a]

	time of primary hydrolysis, h	
monosaccharide	0	0.5
rhamnose	0.63	0.66
arabinose	7.58	7.68
xylose	62.34	61.48
mannose	0.00	0.10
galactose	1.01	0.99
glucose	1.86	2.87

[a] Results are the means of duplicate analysis and are expressed as grams of sugar/100 g of dry matter. Secondary hydrolysis is performed in 2 N H_2SO_4 at 100 °C for 120 min.

Increasing 4 times the dichloromethane volume proposed by Blakeney et al. (1983), the recovery of dichloromethane is complete (Figure 3) and all alditol acetates are completely extracted (100.5 ± 3.6%). On the other hand, the recovery of nonvolatile products is also slightly increased (Figure 3). During chromatographic analysis, these compounds (mainly DMSO and 1-methylimidazole) lead to solvent trailing, which interferes with the first alditol acetate peaks and shortens column life. There is a preferential partition of these compounds toward the water phase. The possibility of eliminating them has been studied by increasing the amount of water used to decompose the excess acetic anhydride, by washing the dichloromethane extract with water. A 2- or 3-fold increase of the water volume, added prior to extraction, decreases proportionately the volumes of nonvolatile compounds recovered in dichloromethane extract. Alditol acetate recoveries, respectively, 97.7 ± 0.5% and 93.0 ± 0.4%, are measured by including internal standard (inositol hexaacetate) dissolved in dichloromethane added after solvent evaporation. Then, in order to obtain a complete extraction of alditol acetates with a low proportion of nonvolatile compounds, the respective volumes of acetic anhydride, water, and dichloromethane required are 1:5:2. After one, two, or three water washings of the dichloromethane extract, alditol acetate recovery, respectively, 98.4 ± 0.8%, 97.6 ± 0.6%, and 98.4 ± 0.8%, is not affected. After the second washing, residual quantities are low enough to reduce solvent trailing and to allow satisfactory chromatographic conditions (Figure 4).

By increasing the volume of dichloromethane used for extraction, alditol acetates are diluted. The possibility of increasing the amounts of aldoses submitted to reduction and acetylation has been investigated. Various aliquots (0.2, 0.4, 0.6, 0.8, 1.0 mL) of a solution containing equal amounts (1 g/L) of six monosaccharides are reduced and acetylated. The relationship between the monosaccharide amounts and the detector response factor is linear up to an aliquot of 0.6 mg for rhamnose, 0.8 mg for glucose, galactose, and mannose, and 1 mg for xylose and arabinose. The occurrence of an incomplete reduction or acetylation of rhamnose and glucose has already been observed (Blakeney et al., 1983; Selvendran et al., 1979). This effect can be overcome by increasing 3-fold the hydrolysate aliquot volume. Nevertheless, under these conditions, the response factor of each monosaccharide is lower and generally less repeatable (Table VI). So, in the recommended procedure, 0.2 mL of aldose mixture or cell wall hydrolysate is submitted to reduction and acetylation. To increase sensitivity of the chromatographic analysis, dichloromethane extracts are concentrated five times by evaporation under vacuum at 40 °C.

Separation of seven alditol acetates is obtained within 20 min on a packed column (3% SP 2340) or within 12 min

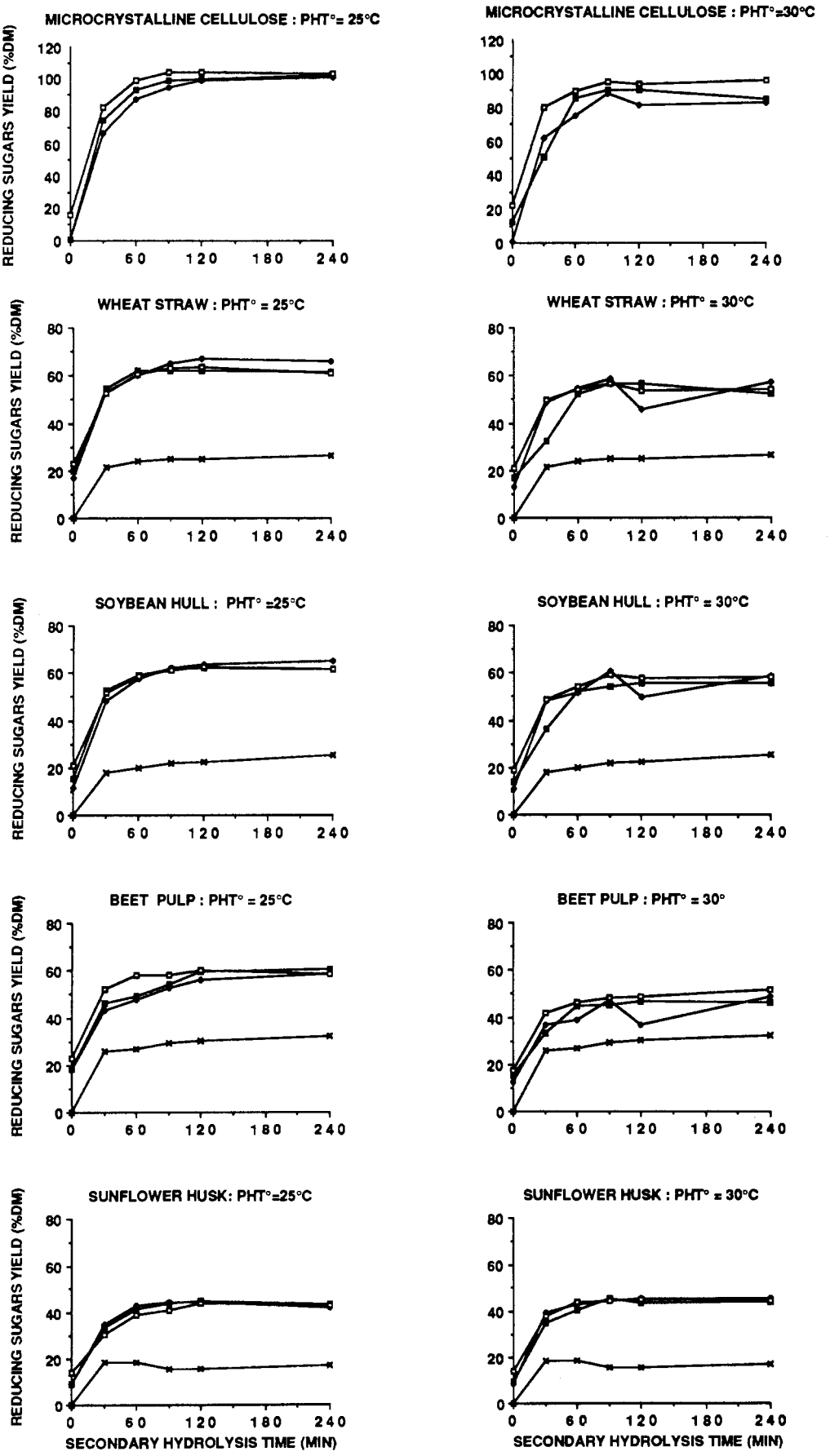


Figure 2. Effect of time of two-stage hydrolysis. Reducing sugar yields as glucose after primary hydrolysis performed in 72% (w/w) sulfuric acid followed by secondary hydrolysis in 2 N acid at 100 °C. Primary hydrolysis time: (×) 0 min; (●) 15 min; (■) 30 min; (□) 60 min. PHT° = primary hydrolysis temperature.

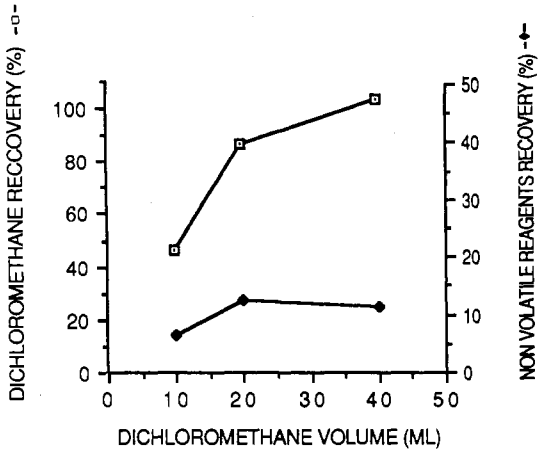


Figure 3. Effect of the dichloromethane volume used for alditol acetate extraction on the rate of the dichloromethane and nonvolatile reagents recovered (10 mL of dichloromethane added corresponds to the Blakeney et al. procedure).

on a capillary column (DB 225). The resolution on DB 225 is comparable to that obtained on BP 75 (WCOT column, 6 × 0.2 mm (i.d.)) or SILAR 10C (SCOT column, 28 m × 0.5 mm (i.d.)), used by Blakeney et al. (1982, 1983).

Comparison of Total Procedure Applied to Various Plant Materials. The sugar content of seven plant materials (beet pulp, citrus pulp, copra meal, wheat bran, wheat straw, soybean hull, sunflower husk) was determined by the optimized procedure described in the present paper (method 1) and also by adapting Saeman et al. (1954) hydrolysis (method 2), followed by sugar gas–liquid chromatography whose conditions are described in the Experimental Section. Results of both determinations are compared: The correlation coefficients are equal or superior to 0.99 for all sugars but for rhamnose, whose concentration is too low for an accurate determination (Table VII). The linear regression slope is not significantly different from 1, and values of intercepts are very near to zero. Both methods give very similar results.

Table VI. Detector Response Factor ($\times 10^{-3}$) for 0.2 mL of Monosaccharide Mixed Standard Hydrolysis Solutions (0.563 g/L of Each Monosaccharide)[a]

	aliquot of monosaccharide std soln, mL	
alditol acetate	0.6	0.2
rhamnitol	1101 ± 54	1132 ± 58
arabinitol	921 ± 42	1000 ± 31
xylitol	904 ± 42	980 ± 28
mannitol	960 ± 41	1096 ± 28
galactitol	934 ± 45	1078 ± 28
glucitol	986 ± 31	1079 ± 35

[a] Each mixture of monosaccharides (0.563 g/L of each monosaccharide) is reduced with 2 mL of sodium borohydride solution and then acetylated by 4 mL of acetic anhydride in the presence of 0.4 mL of 1-methylimidazole. The detector response factors are calculated with inositol hexacetate added as a internal standard.

CONCLUSIONS

The proposed method allows an accurate determination of nonstarch polysaccharides in a relatively short time, irrespective of the cell wall substrate. The analytical error (σ/n) in the determination of monosaccharides from cell wall materials is 3.1% (n = 7) for sugars whose amount is higher than 1% of the dry matter. However, the primary hydrolysis temperature, which is the most significant parameter, has no influence on the hydrolysis of highly lignified materials, such as sunflower husk. The recom-

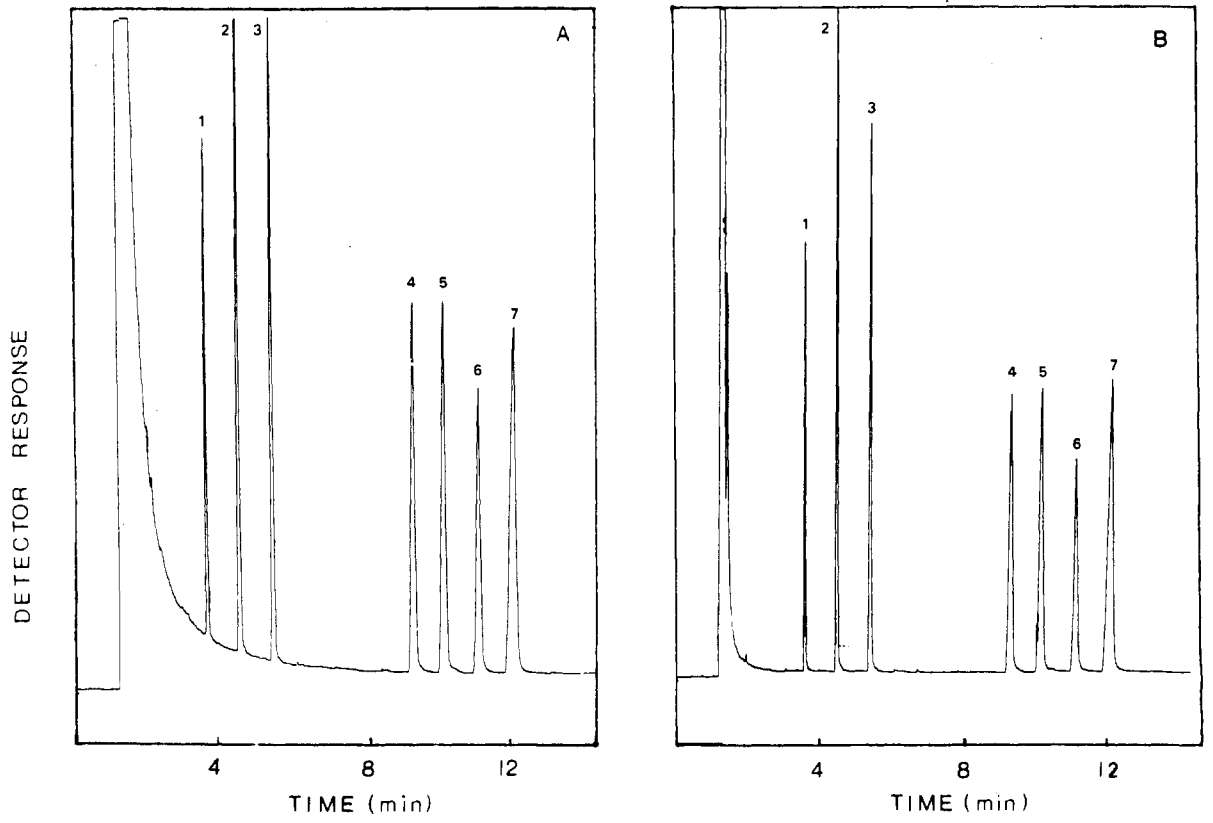


Figure 4. Separation of alditol acetates by gas–liquid chromatography on DB 225 silica capillary column. Chromatogram A: alditol acetates only extracted by dichloromethane. Chromatogram B: alditol acetates extracted by dichloromethane followed by two water washings. In both cases, solvent extracts are evaporated to dryness and residues are taken up in 1 mL of dichloromethane. Conditions: volume injected, 1.5 μL; column temperature, 220 °C; carrier gas (hydrogen) flow rate, 1.1 mL min^{-1}. Peaks: (1) rhamnitol, (2) arabinitol, (3) xylitol, (4) mannitol, (5) galactitol, (6) glucitol, (7) inositol.

Table VII. Regression Coefficient between Sugars of Various Plant Cell Wall Materials, Determined by Two Gas-Liquid Chromatographic Methods[a]

sugar	lin regressn slope	intercept	correln coeff
rhamnose	1.015	+0.153	0.971
arabinose	1.006	−0.652	0.997
xylose	0.967	+0.033	0.996
mannose	1.067	−0.028	1.000
galactose	1.064	−0.093	0.989
glucose	1.012	+0.460	0.988

[a] Method 1: Substrates are dissolved in 72% (w/w) sulfuric acid at 25 °C for 30 min; hydrolysis is performed in 2 N acid at 100 °C for 120 min. Released monosaccharides are transformed as alditol acetates according to the described procedure and analyzed by gas-liquid chromatography on a DB 225 silica capillary column. Method 2: Substrates are submitted to Saeman hydrolysis followed by hydrolyssi in 2 N acid at 100 °C for 150 min; neutral sugars are estimated by gas-liquid chromatography on an SP 2340 packed column.

mended hydrolysis conditions are suitable for quantitative determination of hemicellulosic monosaccharides without loss during the two-step hydrolysis. The content of some hemicellulosic sugars (xylose, mannose, galactose) can be underestimated after only the hydrolysis in dilute acid: About 27% of wheat straw xylose is not released during that hydrolysis step (unpublished data). But, for determination of glucose originating from noncellulosic sources, hydrolysis in dilute acid is a necessary step. In the case of plant samples containing large amounts of lipids, soluble sugars, starch, or proteins, the removal of these substances by chemical or enzymatic treatment (Schweizer and Würsch, 1979; Selvendran and Du Pont, 1980; Prosky et al., 1985; Brillouet et al., 1988) avoids the underestimation of cell wall sugars or the occurrence of artifacts during acid hydrolysis.

Registry No. Sulfuric acid, 7664-93-9; cellulose, 9004-34-6; hemicellulose, 9034-32-6; lignin, 9005-53-2; L-rhamnose, 3615-41-6; D-arabinose, 10323-20-3; D-xylose, 58-86-6; D-mannose, 3458-28-4; D-galactose, 59-23-4; D-glucose, 50-99-7; xylan, 9014-63-5.

LITERATURE CITED

Abou-State, M. A.; Add El-Megeid, F. F.; Helmy, S. A. Some aspects of the structure and accessibility of bagasse cellulose as related to prehydrolysis. *Holzforschung* **1983**, *37*, 293–296.

Albersheim, P.; Nevins, D. J.; English, P. D.; Karr, A. A. method for the analysis of sugars in plant cell-wall polysaccharides by gas liquid chromatography. *Carbohydr. Res.* **1967**, *5*, 340–345.

Bittner, A. S.; Harris, L. E.; Campbell, W. F. Rapid N-methylimidazole catalyzed acetylation of plant cell wall sugars. *J. Agric. Food Chem.* **1980**, *28*, 1242–1245.

Blakeney, A. B.; Harris, P. J.; Henry, R. J.; Stone, B. A. Gas chromatography of alditol acetates on a high-polarity bounded phase vitreous-silica column. *J. Chromatogr.* **1982**, *249*, 180–182.

Blakeney, A. B.; Harris, P. J.; Henry, R. J.; Stone, B. A. A simple and rapid preparation of alditol acetates for monosaccharide analysis. *Carbohydr. Res.* **1983**, *113*, 291–299.

Brillouet, J. M.; Rouau, X.; Hoebler, C.; Barry, J. L.; Carré, B.; Lorta, E. A new reference method for determination of insoluble cell walls and soluble nonstarchy polysaccharides from plant materials. *J. Agric. Food Chem.* **1988**, *36*, 969–979.

Carré, B.; Brillouet, J. M. Yield and composition of cell wall residues isolated from various feedstuffs used for non-ruminants farm animals. *J. Sci. Food Agric.* **1986**, *37*, 341–351.

Caulfield, D. F.; Moore, W. E. Effect of varying crystallinity of cellulose on enzymatic hydrolysis. *Wood Sci.* **1974**, *6*, 375–379.

Connors, K. A.; Pandit, N. K. N-methylimidazole as catalyst for analytical acetylation of hydroxy compounds. *Anal. Chem.* **1978**, *50*, 1542–1545.

Dutton, G. G. S. Gas liquid chromatography. In *Advances in Carbohydrate Chemistry and Biochemistry*; Tipson, R. S., Horton, D., Eds.; Academic: New York, 1973; Vol. 22, pp 11–160.

Englyst, H. N.; Cummings J. H. Simplified method for the measurement of total non-starch polysaccharides by gas-liquid chromatography of constituent sugars as alditols acetates. *Analyst* **1984**, *109*, 937–942.

Feather, M. S.; Harris, J. F. Dehydration reactions of carbohydrates. In *Advances in Carbohydrate Chemistry and Biochemistry*; Tipson, R. S.; Horton, D., Eds.; Academic: New York, 1973; Vol. 22, pp 161–224.

Fengel, D.; Wegener, G. Hydrolysis of polysaccharides with trifluoroacetic acid and its application to rapid wood and pulp analysis. In *Hydrolysis of Cellulose: mechanisms of enzymatic and acid catalysis*; Brown, R. D.; Jurasek, J. L., Eds.; Advances in Chemistry Series 181; American Chemical Society: Washington, DC, 1979; Chapter 7, pp 145–158.

Ford, C. W. Semimicro quantitative determination of carbohydrates in plant material by gas-liquid chromatography. *Anal. Biochem.* **1974**, *57*, 413–420.

Goering, H. K.; Van Soest, P. J. *Forage Fiber Analysis*; Agricutural Handbook No. 379; USDA—ARS, U.S. GPO: Washington, DC, 1970; p 20.

Graham, H.; Hesselman, K.; Aman, P. The influence of wheat bran and sugar beet pulp on digestibility of dietary components in cereal-based pig diet. *J. Nutr.* **1986**, *116*, 242–251.

Hardell, H. L.; Theander, O. Quantitative determination of carbohydrates in cellulosic materials-losses as sulfates. *Svenk Papperstidn.* **1970**, *73*, 291–293.

Hardt, H.; Lamport, D. T. A. Hydrogen fluoride saccharification of cellulose and xylan: isolation of α-D-glucopyranosyl fluoride and α-D-xylopyranosyl fluoride intermediates, and α-D-1,6-anhydro-β-D-glucopyranose. *Phytochemistry* **1982**, *21*, 2301–2303.

Harris, P. J.; Blakeney, A. B.; Henry, R. J.; Stone, B. A. Gas chromatographic determination of the monosaccharide composition of plant cell wall preparations. *J. Assoc. Off. Anal. Chem.* **1988**, *71*, 272–275.

Hoffman, W. S. A rapid photoelectric method for the determination of glucose in blood and urine. *J. Biol. Chem.* **1937**, *12*, 51–53.

Mason, B. S.; Slover, H. T. A gas chromatographic method for the determination of sugars in foods. *J. Agric. Food Chem.* **1971**, *19*, 551–554.

Melcion, J. P.; Delort-Laval, J. Méthodes objectives d'appréciation des caractéristiques physiques des aliments composés. *Ind. Aliment. Anim.* **1979**, *325*, 7–24.

Millet, M. A.; Effland, M. J.; Caulfield, D. F. Influence of fine grinding on the hydrolysis of cellulosic materials-Vs enzymatic. In *Hydrolysis of cellulose: mechanisms of enzymatic and acid catalysis*; Brown, R. D., Jurasek, J. L., Eds.; Advances in Chemistry Series 181; American Chemical Society: Washington, DC, 1979; Chapter 4, pp 71–89.

Montreuil, J.; Spik, G.; Dumaisnil, J.; Monsigny, M. Procédés de détermination de la composition en oses et osides libres et combinés. *Bull. Soc. Chim. Fr.* **1965**, 239–254.

Nyman, M. N.; Asp, N. G. Fermentation of dietary fibe components in the rat intestinal tract. *Br. J. Nutr.* **1982**, *47*, 357–366.

Oades, J. M. Gas-liquid chromatography of alditol acetates and its application to the analysis of sugars in complex hydrolysates. *J. Chromatog.* **1967**, *28*, 246–252.

Paice, M. G.; Jurasek, L.; Desrochers, M. Simplified analysis of wood sugars. *TAPPI* **1982**, *65*, 103–106.

Philip, B.; Jacopian, V.; Loth, F.; Hirte, W.; Schulz, G. Influence of physical structure on the thermohydrolytic, hydrolytic and enzymatic degradation of cellulose. In *Hydrolysis of cellulose: mechanism of enzymatic and acid catalysis*; Brown, R. D., Jurasek, J. L., Eds.; Advances in Chemistry Series 181; American Chemical Society: Washington, DC, 1979; Chapter 6, pp 128–142.

Prosky, L.; Asp, N. G.; Furda, I.; Devries, J. W.; Schweizer, T. F.; Harland, B. F. Determination of total dietary fiber in foods and food products: collaborative study. *J. Assoc. Off. Anal. Chem.* **1985**, *67*, 677–679.

Saeman, J. F.; Moore, W. E.; Mitchell, R. L.; Millet, M. A. Techniques for the determination of pulp constituents by quantitative paper chromatography. *TAPPI* **1954**, *37*, 336–343.

Schweizer, T. F.; Würsch, P. Analysis of dietary fibre. *J. Sci. Food Agric.* **1979**, *30*, 613–619.

Selvendran, R. R.; Du Pont, M. S. Simplified methods for the preparation and analysis of dietary fiber. *J. Sci. Food Agric.* **1980**, *31*, 1173–1182.

Selvendran, R. R.; March, J. F.; Ring, S. G. Determination of aldoses and uronic acid content of vegetable fiber. *Anal. Biochem.* **1979**, *96*, 282–292.

Slavin, J. L.; Marlett, J. A. Evaluation of high-performance liquid chromatography for the measurement of the neutral saccharides in neutral detergent fiber. *J. Agric. Food Chem.* **1983**, *31*, 467–471.

Snedecor, G. W.; Cochran, W. G. *Statistical Analysis*, 6th ed.; Iowa State University Press: Ames, IA, 1979.

Theander, O.; Aman, P. Analysis and chemical characterization of water-soluble and water-insoluble dietary fibres. *Swed. J. Agric. Res.* **1979**, *9*, 97–106.

Thomart, Ph.; Marcoen, J. M.; Desnons, P.; Foucart, M.; Paquot, M. Etude comparative de l'hydrolyse par voie acide de la cellulose. *Holzforschung* **1983**, *37*, 173–178.

Turvey, J. R. Sulfates of the simple Sugars. In *Advances in Carbohydrates Chemistry and Biochemistry*; Wolfrom, M. M. L., Ed.; Academic: New York, 1965; Chapter 20, pp 183–218.

Received for review October 26, 1987. Revised manuscript received July 27, 1988. Accepted August 15, 1988.

Application of Gas Chromatography/Matrix Isolation/Fourier Transform Infrared Spectrometry to the Identification of Glucosinolates from *Brassica* Vegetables

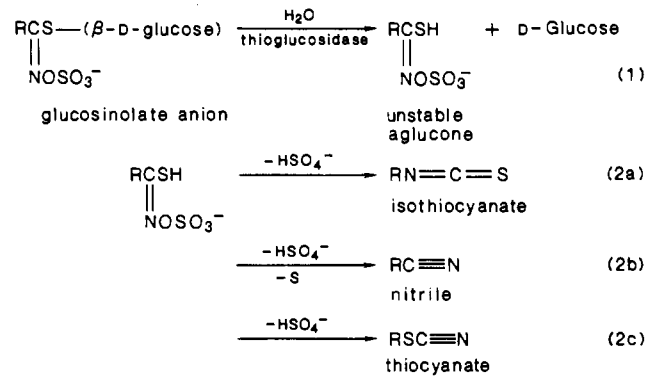
Magdi M. Mossoba,* G. John Shaw,[1] Denis Andrzejewski, James A. Sphon, and Samuel W. Page

There is limited epidemiologic evidence suggesting that ingestion of *Brassica* vegetables may be associated with reduced risk of some cancers of the alimentary tract, and a number of *Brassica* constituents are known to inhibit carcinogenesis in laboratory animals. The components that are responsible for the observed protective activity and the effects of processing on them have not yet been established. An analytical method using capillary gas chromatography/matrix isolation/Fourier transform infrared spectroscopy (GC/MI/FT-IR) has been developed for characterizing a series of intact glucosinolates extracted from Brussels sprouts and rutabaga (swede). Highly resolved gas chromatograms and diagnostic MI/FT-IR spectra were obtained for different glucosinolate analogues with subtle structural differences.

Glucosinolates [RC(=$NOSO_3^-$)—S—(β-D-glucose)] occur naturally in certain families of dicotyledonous angiosperms, including edible crops in the genus *Brassica* of the Cruciferae family (Fenwick et al., 1983; Heaney and Fenwick, 1987). The breakdown products of glucosinolates possess pungent odors and biting tastes. These properties in *Brassica* vegetables such as mustard and horseradish make them desirable as condiments and relishes and in salads (e.g., radish and watercress). The nutritional value of commonly consumed Brassicas, such as cabbage, cauliflower, or Brussels sprouts, lies primarily in their high content of dietary fibers and vitamins A and C (Fenwick et al., 1983).

Some glucosinolate decomposition products have been found to inhibit the neoplastic effects of certain carcinogens (National Research Council, 1982) as well as to possess antifungal and antibacterial activities. On the other hand, adverse effects due to the occurrence of glucosinolates in feed, arising from the use of rapeseed or mustard, have been documented (Heaney and Fenwick, 1987); undesirable consequences include cytotoxicity and the tainting of poultry eggs and dairy milk. Coupled with low dietary iodine, glucosinolates have goitrogenic activity in humans.

Because of recommendations for the increased consumption of cruciferous vegetables related to their anticarcinogenicity (National Research Council, 1982), interest has increased in the physiological activities (Heaney and Fenwick, 1987) of the various aglucone products resulting from the enzymatic hydrolysis of glucosinolates (reactions 1 and 2) during food processing, cooking, or ingestion.



The myrosinase enzyme responsible for the hydrolysis of glucosinolates is now considered to have only thioglucosidase activity. The resulting unstable aglucone (reaction 1) undergoes Lossen rearrangement (Ettlinger and Lundeen, 1957) to yield various products (reaction 2), depending on the nature of the side chain (R), among other factors.

Many methods have been used to monitor both total and individual glucosinolate content of Brassicas (McGregor et al., 1983) including liquid chromatography/mass spectrometry (Hogge et al., 1987) and packed (Christensen et al., 1982) and capillary column gas chromatography/mass spectrometry (GC–MS) (Hiltunen et al., 1980; Sosulski and

Division of Contaminants Chemistry, Food and Drug Administration, 200 C Street SW, Washington, D.C. 20204.

[1] Present address: Biotechnology Division, DSIR, Private Bag, Palmerston North, New Zealand.