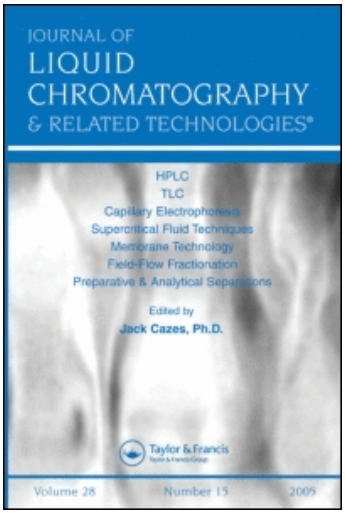

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High Performance Liquid Phase Separation of Glycosides. II. Capillary Electrophoresis of the Fluorescently Labeled Acid Hydrolysis Products of Glucosinolates—Profiling of Glucosinolates in White and Red Cabbages†

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HIGH PERFORMANCE LIQUID PHASE SEPARATION OF GLYCOSIDES. II. CAPILLARY ELECTROPHORESIS OF THE FLUORESCENTLY LABELED ACID HYDROLYSIS PRODUCTS OF GLUCOSINOLATES—PROFILING OF GLUCOSINOLATES IN WHITE AND RED CABBAGES†

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ABSTRACT

In this series of papers, we are reporting on the rapid and sensitive detection of glycosides by high performance liquid phase separation methods such as high performance liquid chromatography (HPLC), capillary electrophoresis (HPCE), and capillary electrochromatography (CEC). In this report, which is the second in this series, we are describing the detection of glucosinolates via their acid hydrolysis degradation products. The rationale behind this approach is the lack of authentic standards, a fact that makes the routine analysis of glucosinolates rather difficult. Upon acid hydrolysis, glucosinolates hydrolyze, among other things, to carboxylated compounds that are reflective of the individual glucosinolates.

The carboxylated acid hydrolysis products can be readily labeled with fluorescent tags through their carboxylic acid groups. This involved the reaction of the hydrolysis products with 7-aminonaphthalene-1,3-disulfonic acid (ANDSA) fluorescing tag in the presence of carbodiimide. This condensation reaction produced stable and fluorescing derivatives with high yields and exhibited little or no side products. The fluorescent derivatives were separated and detected by HPCE-laser induced fluorescence (HPCE-LIF) at low levels, i.e., 2.5 x 10^{-8} M. In one approach, capillary zone electrophoresis in the presence of the neutral n-octyl-β-D-glucoside (OG) micelle provided baseline separation of the ANDSA derivatized acid hydrolysis products when 50 mM sodium phosphate, pH 6.5, containing 50 mM OG was used as the running electrolyte.

In a second separation approach, the derivatives were separated by micellar electrokinetic capillary chromatography (MECC) in the presence of in situ charged micelles composed of OG-borate complexes. Due to the flexibility of the in situ charged micellar system, the selectivity of the separation could be readily manipulated by varying the surfactant concentration. The developed methodologies applied readily to the detection of glucosinolates in real world samples, namely white and red cabbage extracts.

INTRODUCTION

Glucosinolates are anionic thioglucosides whose structures vary mainly by the nature of the side chain (R-group) which can be aliphatic or aromatic, see Fig. 1. There are currently more than 100 known glucosinolates.[1] All species of the Cruciferae family including forages, rapeseed, cole crops, mustards, and horseradish contain one or more glucosinolates, occurring throughout the plant, in root, stem, leaf, and seed.[1-4] Several of these glucosinolate-containing taxa, possess vast economic importance. The major horticultural crop containing glucosinolates is cabbage, and it is consumed throughout the world including the USA by millions of tons.

Glucosinolates are classified among the most important naturally occurring toxins in plant foodstuffs.[3] Glucosinolates are associated with various flavor, off-flavor, and antinutritive effects and are involved in goitrogenic activity.[5]

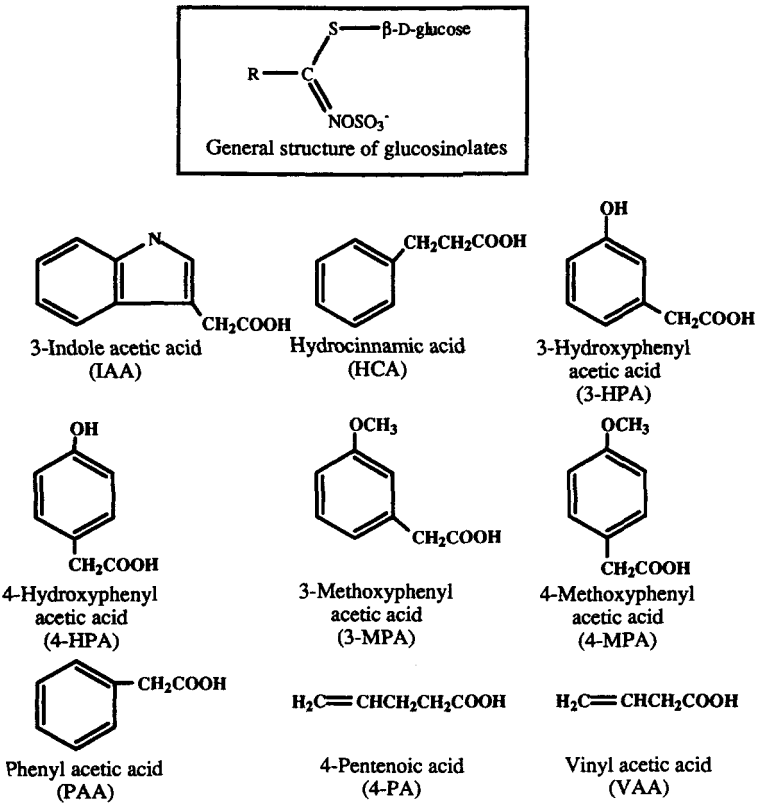


Figure 1. General structure of glucosinolates and structures of the standard acid hydrolysis products used in this study. Characters in parentheses represent the compounds abbreviation used in this report.

The determination of glucosinolates has been performed by various analytical separation methods including gas chromatography (GC), GC-mass spectrometry (GC-MS),[6,7] high performance liquid chromatography (HPLC),[8,9] and HPLC-MS.[10] Due to their ionic nature, glucosinolates are not directly amenable to GC and require a precolumn derivatization. High performance liquid phase separation methods such as HPLC and HPCE are therefore more readily applicable to the analysis of glucosinolates. In fact, HPLC using reversed-phase chromatography has been shown as an efficient method for the isolation of intact glucosinolates.[11,12] Very recently, high performance capillary electrophoresis (HPCE) has become an important tool in the analysis of glucosinolates. Using micellar electrokinetic chromatography (MECC),

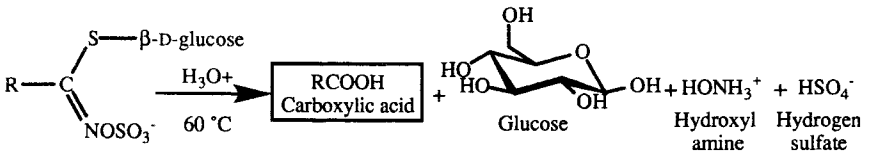


Figure 2. Reaction scheme depicting the products produced from the acid hydrolysis of a glucosinolate.

Sørensen et al. effectively separated eleven intact glucosinolates from double-low rapeseed.[13] In another report from the same research group, HPCE proved useful in the determination of indolyl glucosinolates and their transformation products.[14]

One existing major difficulty in the analysis of glucosinolates is the lack of authentic standards. In fact, among the 100 known glucosinolates, only one of them, namely sinigrin (i.e., allylglucosinolate) and gluconasturtiim are available from commercial sources as relatively pure standards. This makes routine analysis of glucosinolates in food and feed very difficult to achieve. To overcome this difficulty, glucosinolates can be readily broken down by chemical and enzymatic means into fragments reflective of the individual glucosinolates. Several of these degradation products are available as relatively pure standards or can be synthetized readily and more easily than the parent glucosinolates. Usually, glucosinolates occur in food and feed together with a hydrolyzing enzyme called myrosinase. When part of a given plant is masticated or crushed, the myrosinase comes in contact with the glucosinolates, hydrolyzing them into fragments that are reflective of the parent glucosinolates.[1,2] There are also chemical means that can be used to degrade glucosinolates.[15] For instance, acid hydrolysis of glucosinolates yields carboxylated degradation products seen in Fig. 2. Although these carboxylated degradation products can be used for the determination of individual glucosinolates, some of them lack strong chromophores needed for adequate UV detection (see Fig. 1). Thus, there is a need to develop a method for the rapid and sensitive analysis of glucosinolates through their degradation products. Usually, the degradation products offer reactive sites for the attachment of fluorescent tags.

The aim of this report, which is a continuation to our previous investigation,[16] is to introduce a selective precolumn derivatization for the sensitive determination of glucosinolates via their acid hydrolysis degradation products. This involves the targeting of the degradation products in real world samples with a specific reagent, which converts the degradation products to

fluorescently labeled derivatives independently of the origin of the glucosinolate sample. This should enable the rapid HPCE-laser induced fluorescence (LIF) detection of the parent glucosinolates.

EXPERIMENTAL

Reagents and Materials

The acid hydrolysis standards of the glucosinolates including 4-methoxyphenylacetic acid (4-MPA), 3-methoxyphenylacetic acid (3-MPA), 4-hydroxyphenylacetic acid (4-HPA), phenylacetic acid (PAA), 3-hydroxyphenylacetic acid (3-HPA), hydrocinnamic acid (HCA), 4-pentenoic acid (4-PA), and vinylacetic acid (VAA) were purchased from Aldrich (Milwaukee, WI, USA), while indole-3-acetic acid (IAA) was purchased from Sigma (St. Louis, MO, USA). Structures for the acid hydrolysis product standards are given in Fig. 1. Red and white cabbages were purchased from a local grocery outlet. Octyl-β-D-glucopyranoside (OG) was purchased from Anatrace (Mumee, OH, USA). A 1.0 M solution of 1,3-dicyclohexylcarbodiimide (DCC) dissolved in dichloromethane and 1,1'-binaphthyl-2,2'diylhydrogen phosphate were purchased from Aldrich. The precolumn labeling agent 7-aminonaphthalene-1,3-disulfonic acid (ANDSA) was purchased from TCI America Inc. (Portland, OR, USA). Myrosinase (thioglucosidase, EC 3.2.3.1) used in the enzymatic hydrolysis of glucosinolates was purchased from Sigma Chemical Co. One unit of myrosinase produces 1.0 μmole glucose per min from sinigrin (a glucosinolate) at pH 6.0 and at 25°C.

Capillary Electrophoresis Instruments

Two Beckman P/ACE instruments (Fullerton, CA, USA) were used. They consisted of Models 5510 and 5010 equipped with a diode array detector and an Ominichrome (Chino, CA, USA) Model 3056-8M He-Cd laser multimode, 8 mW at 325 nm, respectively. By use of the instruments thermostating capabilities, the temperature of the capillary was held constant at 25°C. A personal computer and P/ACE Station software were utilized for data handling purposes. The UV detection of the underivatized acid hydrolysis standards was performed at 200 nm. The UV detection of the ANDSA labeled derivatives was performed at 255 nm. A wavelength of 225 nm was selected for the detection of intact glucosinolates. For the LIF detection of the ANDSA derivatives, a fluorescence emission band-pass filter of 380 ± 2 nm and 420 ± 2 nm were purchased from Corion (Holliston, MA, USA). A 360 nm cut-on filter

purchased from Corion was used to reject the laser beam. All experiments were performed in fused-silica capillaries obtained from Polymicro Technology (Phoenix, AZ, USA). The dimensions of the capillaries were 50 cm to the detection window and 57 cm total length with 50 μm internal diameter and 365 μm outer diameter. Samples were pressure injected at 0.034 bar (i.e., 3.5 kPa) for various lengths of time. In between runs, the capillary was rinsed with 0.1 M NaOH, distilled water, and running electrolyte for two, three, and one minute, respectively.

Procedures

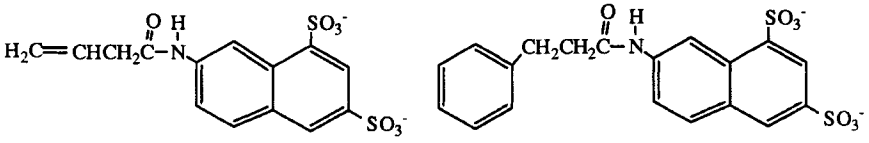
Extraction and acid hydrolysis of glucosinolates from red and white cabbages

The extraction of the glucosinolates from both the white and red cabbages was performed by the following procedures.[15] One leaf of cabbage was carefully removed, weighed, and frozen with liquid nitrogen. The frozen leaf was then placed in a freeze drying apparatus and dried for twenty-four hours. A mortar and pestle that had been previously cooled to -5°C was used to grind the dried cabbage leaf. Immediately following grinding, approximately 25 mL of boiling methanol was pipetted over the ground leaf. The resulting mixture was filtered through a Whatman # 1 filter to remove the solid particulates, with the filtrate containing the intact glucosinolates. In another extraction procedure cabbage leaves (60 g) were placed in a dewar containing liquid nitrogen until freezing. The frozen leaves were then grounded to a fine solid in a Regal Coffee Grinder (Kewaskum, WI). The ground cabbage was then placed in a beaker containing 70 mL of boiling methanol. The methanol-ground cabbage mixture was heated at 65°C for 10 min. After cooling, the resulting mixture was filtered as above to yield the methanol which contained the glucosinolates.

The filtrate (obtained from both types of extraction) was equally split into vials amenable for evaporation via a speed vacuum concentrator and the methanol was removed. A portion of the extract was acid hydrolyzed with 6M HCl.[17] The resulting solution was evaporated by speed vacuum and reconstituted with 2 mL of dry pyridine. A representative reaction scheme for the acid hydrolysis of glucosinolates is illustrated in Fig. 2.

Precolumn derivatization of the acid hydrolysis products of glucosinolates

The standard acid hydrolysis products were tagged according to our published procedure[18-20] by first mixing 2.0 mL of a 2.0×10^{-3} M solution of



VAA-ANDSA HCA-ANDSA

Figure 3. Two representative ANDSA derivatives of the acid hydrolysis products of glucosinolates.

the analyte dissolved in dry pyridine. The appropriate amount of DCC was added to these solutions, followed by the appropriate mass of ANDSA to yield a 20 x 10^{-3} M solution. This was allowed to react overnight, and the pyridine was removed via speed vacuum. The solution was prepared for analysis by redissolving the derivatized analyte with a 50% aqueous-acetonitrile solution.

To derivatize the acid hydrolysis products obtained from the red and white cabbage samples, a slightly different precolumn derivatization procedure was used. Enough DCC was added to the final pyridine solution obtained from the acid hydrolysis procedure to produce a concentration of 20 x 10^{-3} M. To this, the correct mass of ANDSA was added to yield a 0.10 M solution. Upon reaction, the pyridine was evaporated, and the tagged analyte was redissolved with the previously stated acetonitrile solution. This solution was analyzed by CE to profile some of the glucosinolates present in the red and white cabbage leaves.

RESULTS AND DISCUSSION

Precolumn Derivatization, Fluorescence Properties and Limits of Detection

Figure 3 shows two typical derivatives of the acid hydrolysis products with ANDSA. In this derivatization reaction, a stable amide bond is formed by condensation between the amino group of ANDSA and the carboxylic group of the analyte. [21,22] The extent of the precolumn derivatization with ANDSA was assessed by CE using 1,1'-binaphthyl-2,2'diylhydrogen phosphate as the internal standard under the running conditions of Fig. 4. Table 1 lists the yields of this derivatization procedure for some of the acid hydrolysis standards.

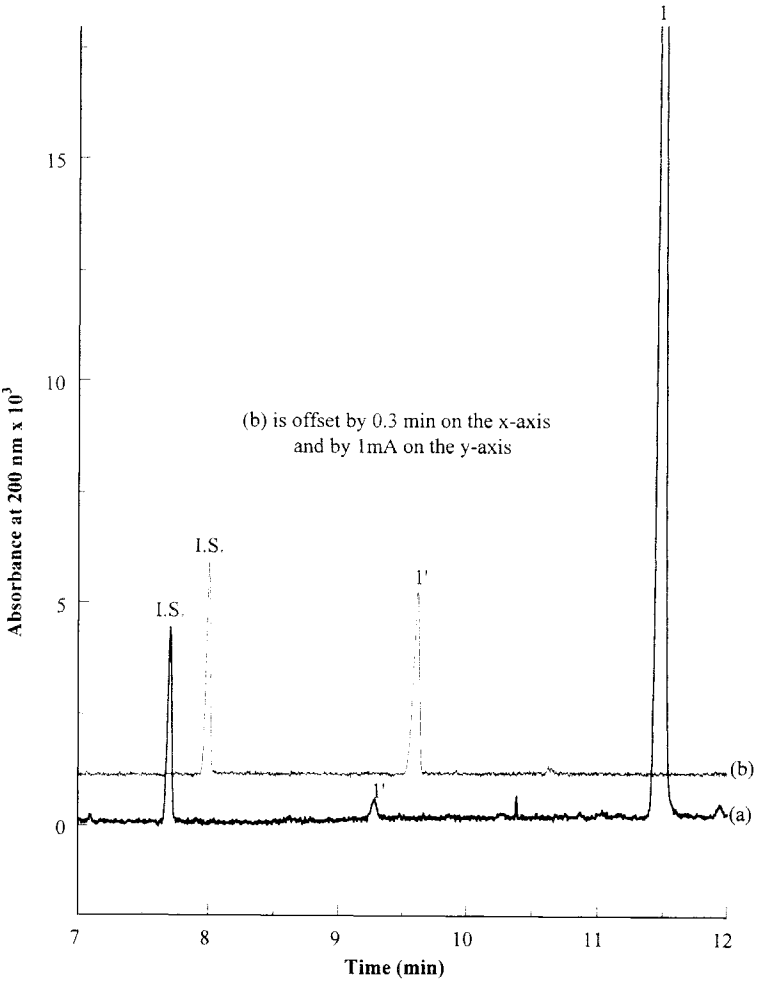


Figure 4. Electropherograms of IAA before and after derivatization with ANDSA. Electrolyte: 50 mM sodium phosphate, pH = 6.5; 15 kV; 25 °C; capillary, fused-silica, l = 50 cm, L = 57 cm, 50 μm i.d.. Solutes: I.S., internal standard; 1', underivatized IAA; 1, IAA-ANDSA derivative.

The derivatization is highly quantitative as indicated by the percent derivatized which ranged from 88% for 4-MPA to a high of 94% for IAA. Figure 4 shows typical electropherograms obtained before and after derivatization for IAA where it can be seen that the amount of remaining

Table 1

Percent Derivatized and Limits of Detection of the Acid Hydrolysis Product Standards of the Glucosinolates*

Analyte	% Derivatized	UV LOD at 200 nm of Underivatized	UV LOD at nm of ANDSA Derivatives (M)	LIF LOD of ANDSA Derivatives (M)
IAA	94	2.9×10^{-5}	2.0×10^{-5}	9.4×10^{-6} 2.4×10^{-5}
4-HPA	93	2.0×10^{-5}	1.0×10^{-5}	1.9×10^{-6}
4-MPA	88	2.0×10^{-5}	1.0×10^{-5}	4.4×10^{-7}
4-PA	91	4.0×10^{-4}	1.0×10^{-5}	8.5×10^{-7}
PAA	88	8.5×10^{-5}	1.8×10^{-5}	2.0×10^{-7}
HCA	93	2.0×10^{-5}	1.0×10^{-5}	2.5×10^{-8}
VAA	--	1.0×10^{-3}	1.0×10^{-5}	4.0×10^{-7}

* For fluorescence detection λ_{ex} = 325 nm (the He/Cd 325 nm laser line) and λ_{em} = 320 nm.

underivatized IAA can hardly be seen. Figure 5 shows the fluorescence spectra obtained for four of the acid hydrolysis product standards labeled with ANDSA. Each of the spectra was obtained with the concentration of each individual derivative constant. This spectral information provided the insight for optimum working conditions for detection with the LIF system. Utilizing this information, the 380 nm band pass filter was chosen. Figure 5 shows that the 325 nm laser excitation source provides an excellent source for the fluorescence of these derivatized analytes. Both the emission and excitation spectra support the data for the LOD studies, see below. The two strongest fluorescing species, namely HCA-ANDSA and VAA-ANDSA, show the most intense emission and excitation spectra.

Table 1 lists the limits of detection that were determined using UV and LIF detection schemes. The LOD for the underivatized analytes closely parallels that for the derivatized analytes. All those containing aromatic groups (e.g., 4-HPA, 4-MPA, PAA, and HCA) allowed adequate UV detection ranging from 8.5×10^{-5} M to 2.0×10^{-5} M. Some of the other hydrolytic products (e.g., 4-PA and VAA) listed in Fig. 1, lack strong chromophoric centers needed for sensitive UV detection. The LIF detection scheme decreased the overall LOD for 4-MPA by almost two orders of magnitude from 2.0×10^{-5}

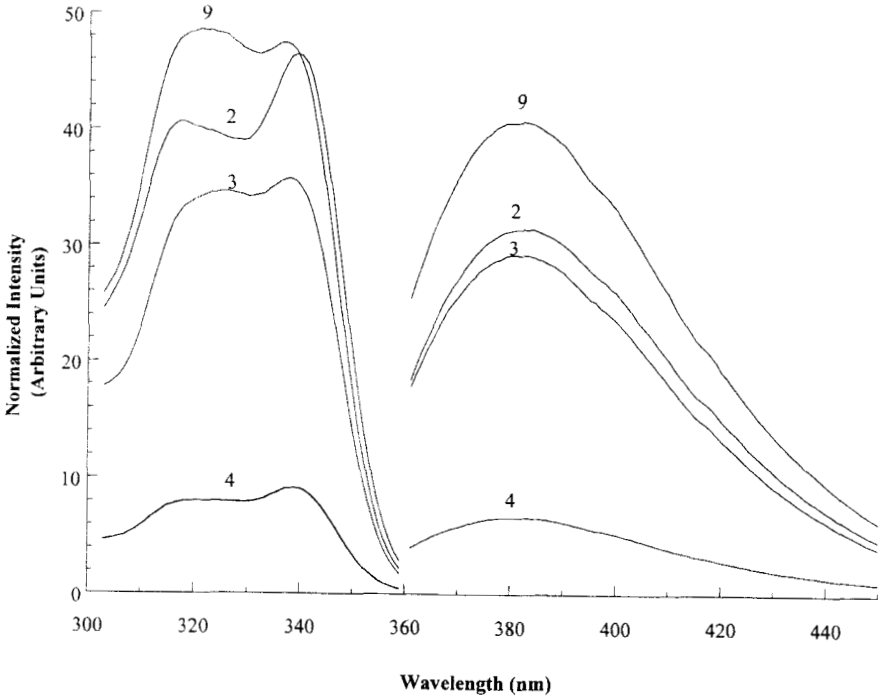


Figure 5. Excitation and emission spectra for four of the ANDSA derivatized acid hydrolysis products. 2, HCA-ANDSA; 3, 4-MPA-ANDSA; 4, 4-HPA-ANDSA; 9, VAA-ANDSA.

M with UV to 4.4 x 10^{-7} M with LIF. The lowest LOD for LIF detection was 2.5 x 10^{-8} M for the HCA derivative. For VAA labeled with ANDSA the LIF LOD was 4.0 x 10^{-7} M compared to that of 1 mM for UV detection. IAA exhibited a high LIF LOD. This can be explained by the fluorescence quenching of the indole ring.[23] The LIF LOD for IAA can be slightly lowered by using a 420 nm band pass filter at the expense of higher LOD's for the remaining analytes.

Capillary Electrophoresis with a Neutral Surfactant System

Using 50 mM sodium phosphate, pH 6.5, as the running electrolyte, a 20 kV applied voltage and a separation distance of 50 cm, the underivatized standard analytes shown in Fig. 1 migrated over a narrow range of time from

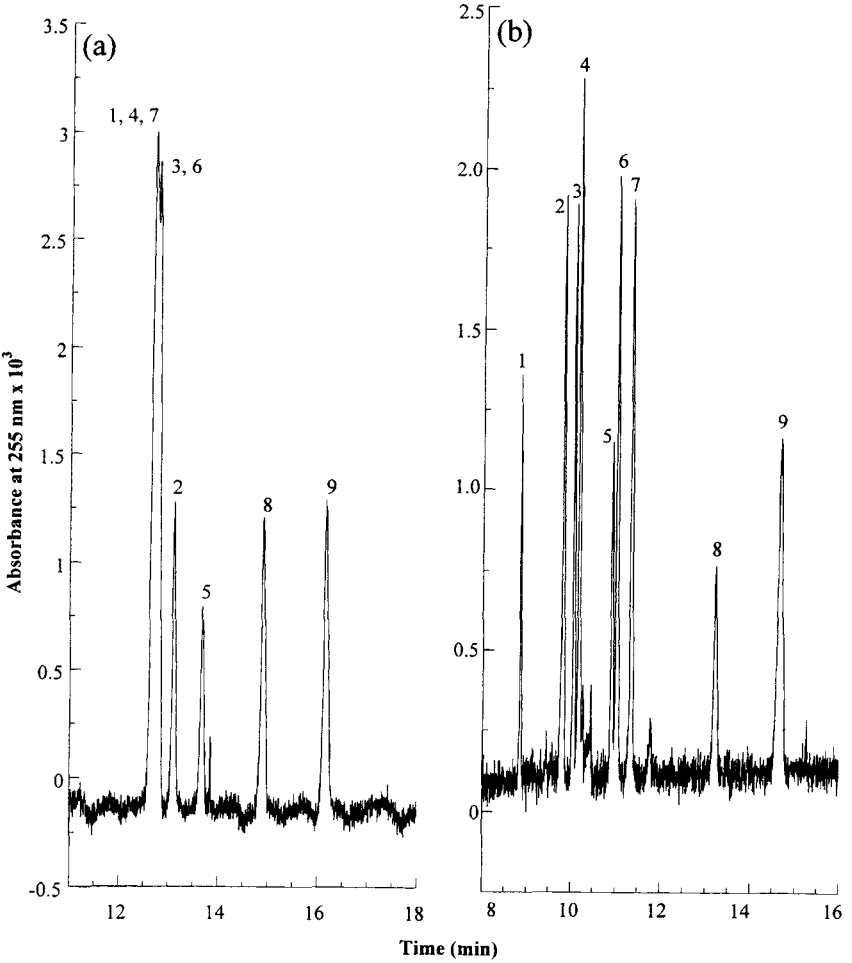


Figure 6. Electropherogram of the ANDSA derivatives of the acid hydrolysis product standards. Electrolytes: (a), 50 mM sodium phosphate, pH = 6.5; (b), same as in (a) containing 50 mM OG. Solutes: 1, IAA; 2, HCA; 3, 3-MPA; 4, 4-MPA; 5, PAA; 6, 3-HPA; 7, 4-HPA; 8, 4-PA; 9, VAA. Other conditions as in Fig. 4.

9.44 to 10.658 min. IAA migrated first (at 9.44 min) separated from HCA (at 9.667 min), while 3-MPA and 4-MPA co-migrated at 9.729 min and also PAA, 3-HPA and 4-HPA co-migrated at 10.658 min. The separation was improved by incorporating 200 mM OG in 100 mM sodium phosphate while keeping other conditions the same.

Table 2

Charge-to-Mass Ratios for the ANDSA Derivatives of the Acid Hydrolyis Product Standards

Analyte	(Charge-to-Mass Ratio) x 10^5
IAA	-3.85
3-MPA, 4-MPA	-3.94
3-HPA, 4-HPA	-4.05
HCA	-4.07
PAA	-4.19
4-PA	-4.53
VAA	-4.68

However, PAA and 4-PA co-migrated at 17.862 min (results not shown). Upon tagging the standard carboxylic acids with ANDSA, the derivatives migrated slower toward the detection end than the underivatized analytes due to the increased charge-to-mass ratios.

A typical electropherogram of the ANDSA labeled analytes run with 50 mM sodium phosphate, pH 6.5 as the running electrolyte is shown Fig. 6a. Although a slight change in the migration order occurred as compared to the underivatized analytes, the complete separation of all ANDSA derivatives solely on the basis of small differences in the charge-to-mass ratio is still not achievable under the specified conditions. This is particularly true for two pairs of positional isomers, namely 4-MPA/3-MPA and 4-HPA/3-HPA. Table 2 lists the charge-to-mass ratio for the nine ANDSA derivatized analytes. Overall, the charge-to-mass ratio for many of the analytes are similar making the separation on this basis alone difficult. As shown in Fig. 6b, the complete separation of the ANDSA derivatives has required the incorporation of OG in the running electrolyte while keeping other conditions the same. The optimum separation conditions for the ANDSA labeled acid hydrolysis product standards was determined via changing the amount of alkylglucoside surfactant (i.e., OG) at a constant sodium phosphate concentration in the running electrolyte or vice versa. For this system the optimum concentration of OG surfactant was 50 mM, and the corresponding optimum sodium phosphate concentration was 50 mM at a pH of 6.5.

In this CZE system just described, the OG surfactant which is functioning as a hydrophobic selector,[18,24] effectively separated all of the nine ANDSA labeled acid hydrolysis product standards. The neutral micelle migrates with

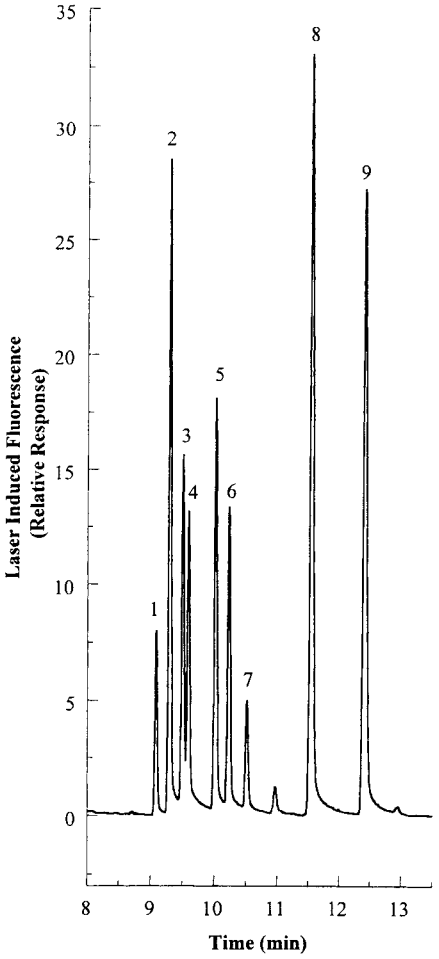


Figure 7. Electropherogram of the ANDSA derivatives of the acid hydrolysis product standards obtained by LIF detection. Electrolytes: 200 mM borate, pH = 8.0, containing 200 mM OG. Solutes as in Fig. 6. Other conditions as in Fig. 4.

the EOF and the most hydrophobic species elute first and the least hydrophobic species elute last.[18,24] Of course, in the presence of the OG neutral micellar phase, the charge-to-mass ratios also influenced the migration of the analytes. For the positional isomers, the separation rested primarily on the hydrophobic character of the solute. Different positioning of the same functional group on a molecule allows positional isomers to exhibit different hydrophobicities. From

Fig. 6b, with the OG micelle functioning as a hydrophobic selector, the positional isomers with the methoxy and hydroxyl groups (3-MPA and 3-HPA) in the meta position elute first, therefore indicating that they are more hydrophobic than the isomers with the ortho substituents.

Capillary Electrophoresis with In-Situ Charged Surfactant System

Electrolyte systems containing in situ charged surfactants are generated by complexing alkylglycoside surfactants via their sugar head groups with borate.[25-31] On this basis, OG can be converted readily to an anionic species in the presence of borate, and at a surfactant concentration above the CMC, an in situ charged micellar system is obtained. Figure 7 shows the separation of the ANDSA labeled acid degradation products using LIF detection and the OG-borate complex as the running electrolyte. Because of the charge introduced dynamically to the OG, the selectivity of the OG-borate electrolyte can be varied over a wide range by varying the charge on the micelle. One way to vary the surface charge density of the OG-borate micelle is through varying the OG concentration.[25-31]

Figure 8 shows the effective electrophoretic mobilities for the derivatized analytes as a function of OG concentration at fixed borate concentration and pH. At low concentrations of OG, many of the analytes exhibit similar effective mobilities which is expected because they are positional isomers. It takes about 200 mM OG in the running electrolyte (i.e., 200 mM borate, pH 8.0) so that all of the analytes exhibit different mobility values.

As the amount of OG was increased from 40 mM to 200 mM, the negative surface charge density of the micelle decreased, a fact that decreased the extent of repulsion between the micelle and analytes thus leading to a stronger association between the negatively charged solutes and micelles.

As can be seen in Fig. 8, with the charged micelle system the optimum concentration of OG was 200 mM in 200 mM borate, pH 8.0. The efficiency for this surfactant system was 298025 compared to 249616 for the efficiency for the CE with the neural micellar system. Thus, inducing repulsion between the solute and micelle could alter the selectivity of the micelle and provide different separation conditions. At 200 mM OG and 200 mM borate, the migration order for the analytes resembles that for the neutral micellar system. By increasing the amount of surfactant, the amount of complexation with borate is reduced, making the MECC system behaves similarly to the CZE system.

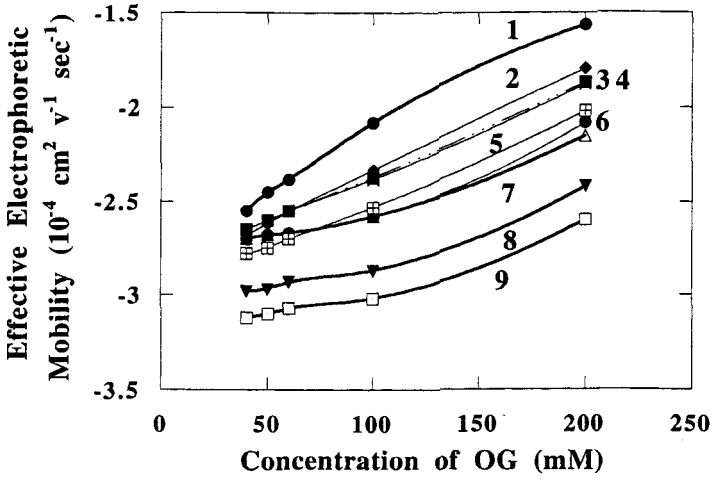


Figure 8. Plots of the effective electrophoretic mobility of the ANDSA derivatives of the acid hydrolysis product standards as a function of the OG concentration in the running electrolyte containing 200 mM borate, pH 8.0. Lines: 1, IAA; 2, HCA; 3, 3-MPA; 4, 4-MPA; 5, PAA; 6, 3-HPA; 7, 4-HPA; 8, 4-PA; 9, VAA. Other conditions as in Fig. 4.

Profiling of Glucosinolates in Cabbages

To assess the utility of the method described here, which consists of detecting glucosinolates via their acid degradation products, it was important first to determine the number of detectable intact glucosinolates in the cabbage. From this piece of information, and using a given detection system, one would expect to find at least a similar number of the side chain degradation products.

A white cabbage sample extracted according to the second procedure described in Experimental was analyzed by CE. Figure 9 shows the electropherograms before and after treatment of the extract with myrosinase. As can be seen in Fig. 9 a, the peak numbered 5 seems to be an acidic solute of nonglucosinolate nature, while the others are glucosinolates because they disappeared from the electropherogram after treatment with myrosinase, compare Fig. 9a and 9b. However, peaks numbered 3 and 4 contain small amounts of negatively charged nonglucosinolate compounds. Peak number 6 is sinigrin as confirmed by spiking the sample with this glucosinolate standard.

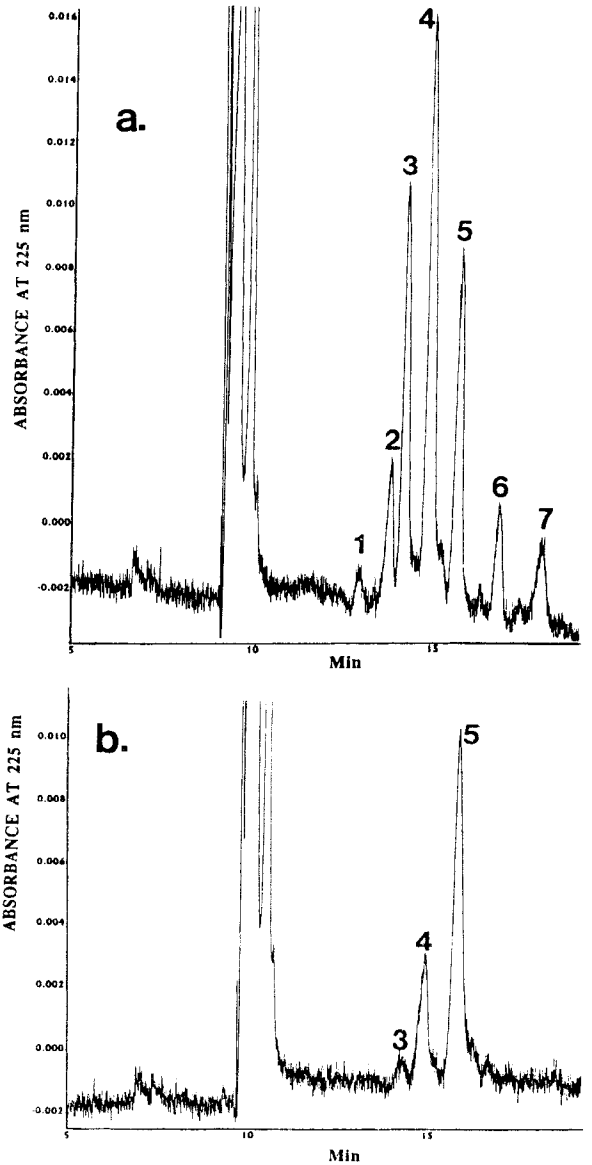


Figure 9. Analysis of white cabbage extract by HPCE-UV before (a) and after myrosinase (b) treatment. Electrolyte: 150 mM phosphate, pH 6.5, containing 100 mM OG; running voltage, +17 kV. Other conditions as in Fig. 4.

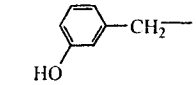
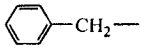
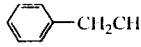
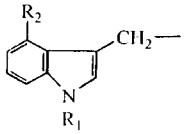
Structure of the R group	Name of the corresponding glucosinolate
$CH_3CH_2CH_2$—	Propylglucosinolate
$CH_3CH_2CH_2CH_2$—	Butylglucosinolate
CH_2=$CHCH_2$—	Allylglucosinolte
CH_2=$CHCH_2CH_2$—	3-Butenylglucosinolate
CH_2=$CHCHCH_2$— (OH)	2(R)-Hydroxy-3-butenylglucosinolate
CH_3SCH_2—	Methylthiomethylglucosinolate
$CH_3SCH_2CH_2CH_2$—	3-Methylthiopropylglucosinolate
$CH_3SOCH_2CH_2CH_2$—	3-Methylsulfinylpropylglucosinolate
$CH_3SOCH_2CH_2CH_2CH_2$—	4-Methylsulfinylbutylglucosinolate
$CH_3SO_2CH_2CH_2CH_2CH_2$—	4-Methylsulfonylbutylglucosinolate
	3-Hydroxybenzylglucosinolate
	Benzylglucosinolate
	Phenethylglucosinolate
	R_1 = R_2 = H, 3-Indolylmethylglucosinolate
	R_1 = OCH_3, R_2 = H, 3-(N-Methoxy)indolylmethylglucosinolate

Figure 10. Structures of the possible glucosinolates in white and red cabbage.

Upon myrosinase digestion of the glucosinolates in the white cabbage sample, the peak corresponding to the EOF has increased in size due to the generation of neutral compounds. The neutral products are most likely to be isothiocyanates (R-N=C=S), thiocyanate (R-S-C°N), and nitriles (R-C°N).[2,3] This indicates that in the white cabbage sample there are at least 6 glucosinolates. According to recent reports on glucosinolate content of cabbages, white cabbage contains large amounts of glucosinolates with three-carbon chains (e.g., allylglucosinolate or sinigrin and 3-methylsulfinylpropylglucosinolate), while red cabbage contains more of the four-carbon glucosinolates (e.g., 4-methylsulfinylbutylglucosinolate). Both cabbages contain 3-indolylmethylglucosinolates (including both 3-indolylmethylglucosinolate and 3-(N-methoxy)indolylmethylglucosinolate),[4] see Fig. 10. Minor glucosinolates are 3-butenylglucosinolates and 2(R)-hydroxy-3-butenylglucosinolate, 2-phenylethylglucosinolate[2], while 3-methylthiopropylglucosinolate, butenylgucosinolate, 4-methylsulfonylbutyl-

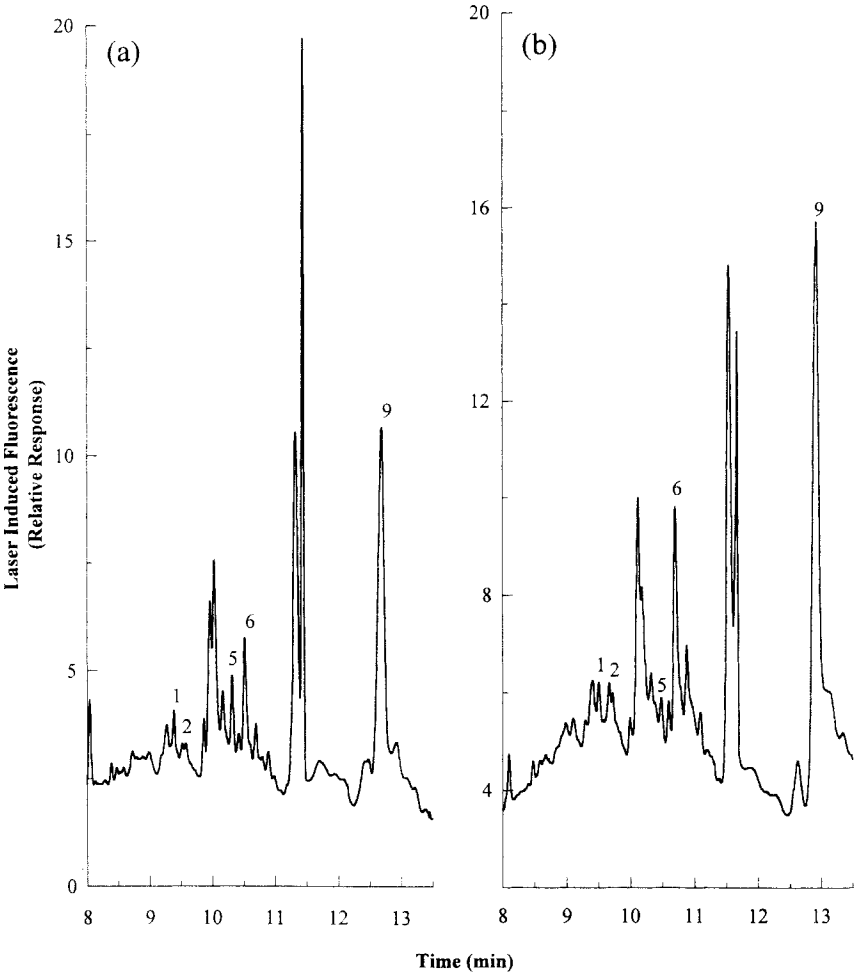


Figure 11. Electropherograms of the ANDSA derivatives of the acid hydrolysis products from the cabbage samples. Electrolytes; 200 mM boric acid containing 200 mM OG. Solutes; red cabbage in (a), white cabbage in (b). Solutes and electrolyte as in Fig. 7. Other conditions as in Fig. 4.

glucosinolate and benzylglucosinolates[2] are present in lesser amounts. Other references have listed the presence of propylglucosinolate, butylglucosinolate, and methylthiomethylglucosinolate.[32] This brings the number of possible glucosinolates that may occur in cabbages to be around 14, see Fig. 10.

However, considerable variation was reported to occur in both individual and total glucosinolate content due to genetic origin and nature of the growing plant as well as the age, the cultural and environmental factors associated with the growth of a particular plant.[32] Returning to Fig. 9 which shows the intact glucosinolates, it is not surprising to see that most of the glucosinolates are hardly detected due to the fact that most of the glucosinolates reported to occur in cabbage have R side chains that lack strong chromophores.

The only glucosinolates that may absorb well in the UV are those as 3-indolylmethylglucosinolates, 2-phenylethylglucosinolate and benzylglucosinolate. But only 3-indolylmethylglucosinolates are abundant to be detected.

Following the extraction, acid hydrolysis and derivatization procedures for the glucosinolates from both the red and white cabbages, the samples were analyzed under the optimum conditions for separation by MECC. LIF detection was used to sensitively and selectively detect the acid hydrolysis products that are reflective of the glucosinolates and for which standards were available.

Figures 11a and 11b are typical electropherograms for the labeled acid hydrolysis products of the glucosinolates from red and white cabbages, respectively. As can be seen in this figure, several major and minor peaks can be detected indicating that cabbages may contain a larger number of glucosinolates than previously reported. LIF detection is more sensitive than any other detection approach previously used.

By spiking the derivatized hydrolyzates from both red and white cabbages with the standard derivatized acids, one can detect the presence of glucosinolates whose degradation products are IAA, HCA, PAA, 3-HPA and VAA. Although the IAA is supposed to be the most abundant glucosinolate, its peak is not intense for both red and white cabbages. This is because the LIF limit of detection of the IAA is the highest, see Table 1.

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