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Short Communication

Direct determination of acetic acid in strongly acidic hydrolysates of chitin and chitin-containing biological products by capillary gas chromatography

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ABSTRACT

Laboratory-made capillary columns with high effectiveness and inertness were used for the determination of N-acetyl groups in chitin-containing biological products by liberation of acetic acid in 4 *M* HCl hydrolysates. The quantitative gas chromatographic analysis is fast, requiring only 2 min, with good precision and accuracy. After a long working period of more than 1000 analyses the capillary column retained its high effectiveness and inertness.

INTRODUCTION

In the analysis of chitin-containing biological products, the determination of N-acetyl groups is based on the measurement of acetic acid liberated after acidic or alkaline hydrolysis of the sample. Holan *et al.* [1] proposed a method for the gas chromatographic (GC) determination of acetic acid in 4 *M* HCl by direct injection into glass columns packed with Porapak Q. The resin had been previously treated according to the method of Mahadevan and Stenroos [2]. In the GC analysis of volatile organic acids Porapak Q columns give satisfactory resolution [1,3].

Progress in capillary GC, however, has allowed inert capillary columns to be constructed that give highly satisfactory resolution and accuracy in the quantitative determination of organic acids [4]. We therefore studied the possibility of using a capillary column for the direct determination of acetic acid in a strongly acidic medium (4 *M* HCl) in the GC determination of acetyl groups in chitin-containing biological materials.

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EXPERIMENTAL

Materials

The experiments were carried out with chitin, chitosan and alkali-insoluble residue derived from the mycelium of the fungus *Humicola lutea*. Crustacean chitin was purchased from Koch-Light (0996H Chitin pract.).

Commercial chitin was used in the preparation of chitosan by hydrolysis with 40% NaOH (ratio 1:10) by boiling for 24 h. Chitosan was separated by filtration, washed with hot distilled water to neutral pH of the filtrates and purified by twice dissolving it in ice-cold 0.1 *M* HCl, followed by titration with 1 *M* NaOH to pH 8.0. The final product was filtered off, washed with hot distilled water and dried by successive washing with absolute ethanol and diethyl ether.

Freeze-dried mycelium of *Humicola lutea* was hydrolysed with 30% NaOH (ratio 1:10) by boiling for 3 h. The alkaline-insoluble residue was separated by filtration and washed with water and organic solvents as described above.

Sample preparation

Deacetylation of the samples was carried out according to the method of Holan *et al.* [1] with 4 *M* HCl. Propionic acid was used as an internal standard.

The glucosamine released after hydrolysis of 15 mg of each sample with 5 ml of 6 *M* HCl in sealed ampoules for 16 h at 105°C, was determined by the spectrophotometric method of Tsuji *et al.* [5].

The percentages of acetic acid and glucosamine were calculated taking into account the ash content of the samples.

Gas chromatography

Duran capillaries were leached with 18% HCl at 170°C for 16 h, rinsed with 1% HCl and dried with nitrogen at 230°C for 4 h. After deactivation with PEG 20M at 280°C for 16 h the capillaries were statically coated with a 0.2-μm film of vinyl-modified PEG 20M (Ohio Valley). The columns had been tested with Grob's test mixture [6].

All analyses were carried out with a Perkin-Elmer Sigma 300 gas chromatograph under the following conditions: column temperature, 120°C; injector and flame ionization detector temperatures, 250°C; carrier gas, nitrogen at a linear velocity of 50 cm/s; and splitting ratio, 1:80. The results obtained were calculated by mean of a Shimadzu CR-1B computing integrator.

RESULTS AND DISCUSSION

The GC determination of acetic acid at concentration below 0.1% is usually accompanied by many disadvantages, including non-linear calibration graphs, inadequate detection limits and the appearance of ghost peaks [7,8]. The acidic hydrolysates used in this investigation represented complex mixtures of organic substances with different physico-chemical properties, where the liberated acetic acid was present at low concentrations (0.01–0.07%). Because of its high efficiency, the capillary column permitted the separation of acetic and propionic acids from the other components of the mixture and allowed quantitative analysis with satisfactory accuracy and precision.

TABLE I

PRECISION AND ACCURACY OF THE GC DETERMINATION OF ACETIC ACID

Concentration (g/l)		Precision, V (%)[a]	Accuracy, A (%)[b]
Model mixture	Calculated value ± standard deviation		
0.80	0.85 ± 0.06	7.1	6.25
0.40	0.42 ± 0.03	7.1	5.0
0.20	0.18 ± 0.02	11.1	10.0
0.10	0.08 ± 0.01	12.5	20.0

[a] $V(\%) = (\bar{x}/S) \cdot 100$ ($n=8$); S = calculated value; $\bar{x}$ = standard deviation. Limit of detection 0.05 g/l.

[b] $$A(\%) = \frac{[CH_3COOH]_{actual} - [CH_3COOH]_{calculated}}{[CH_3COOH]_{actual}} \cdot 100.$$

Data on the accuracy and precision of the GC determination of acetic acid in model mixtures of 4 M HCl with propionic acid as an internal standard are presented in Table I. The concentration of acetic acid in the model mixtures was chosen to be near those usually found in the investigated acidic hydrolysates (below 0.1%). Calibration graphs were generated by plotting the peak-area ratio *versus* the concentration of acetic acid in the concentration range 1.0–0.05 g/l. In all instances the calibration graphs were linear with a correlation coefficient of 0.998 passed through the origin. Values of unknown sample concentrations were determined by comparison with the calibration graph.

The results obtained for acetic acid content in chitin, chitosan and alkali-insoluble residue from the mycelium of *Humicola lutea* are given in Table II. The average values of these results and their standard deviations were calculated for four hydrolysates prepared independently of one another ($n=4$). The molecular ratio between acetic acid and D-glucosamine gave the degree of acetylation of the poly-D-glucosamine in the samples.

The capillary column allowed the rapid analysis of each sample in only 1.5 min. Further, the column showed high stability against strong mineral acids. In analyses of

TABLE II

CONCENTRATION OF ACETIC ACID AND DEGREE OF ACETYLATION OF POLY-D-GLUCOSAMINE IN THE INVESTIGATED SAMPLES

Sample	Acetic acid concentration ± standard deviation (%)	D-Glucosamine (%)	Molecular ratio
Chitin	22.24 ± 0.36	76.82	0.86
Chitosan	2.92 ± 0.57	96.73	0.09
Alkali-insoluble residue from *H. lutea* mycelium	2.20 ± 0.42	15.97	0.41

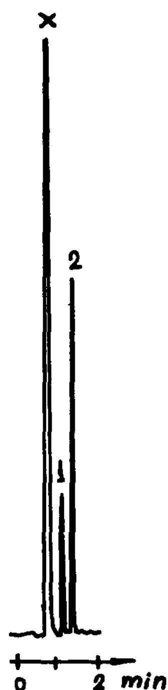

Fig. 1. Chromatogram of acidic hydrolysate from alkali-insoluble residue from *H. lutea* mycelium. Peaks: 1 = acetic acid; 2 = propionic acid; × = volatile impurities.

the acidic hydrolysates it withstood more than 1000 injections, whereas the lifetime of a Porapak Q column was about 150 injections [1].

A chromatogram of the acidic hydrolysate of the fungal alkali-insoluble residue is presented in Fig. 1. The analysis was carried out with a column already used for 1200 injections. This chromatogram illustrates the satisfactory resolution of acetic and propionic acids and the short time needed for analysis.

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