HYDROXYETHYL DERIVATIVES OF THE AMYLOPECTIN STARCH FROM WAXY MAIZE

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Recently, interest in starches and their chemical modifications has risen in connection with the appearance of reports of the successful use of them for medical purposes [1]. It has been established that hydroxyethyl derivatives of starch (hydroxyethylstarch) with a degree of substitution close to unity are, to a certain degree, resistant to enzymatic hydrolysis in the blood circulation system and it is possible to obtain from them a preparation corresponding to a group of the requirements set for blood substitutes with an antishock action [2].

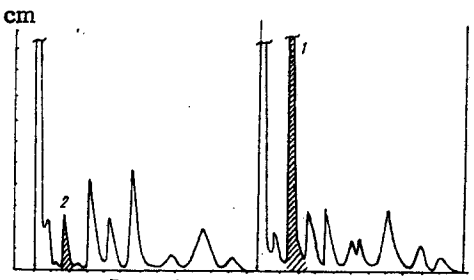

Fig. 1. Chromatograms of the products of the pyrolysis of hydroxyethylstarch (1) and of amylopectin starch (2). The acetaldehyde peak is hatched.

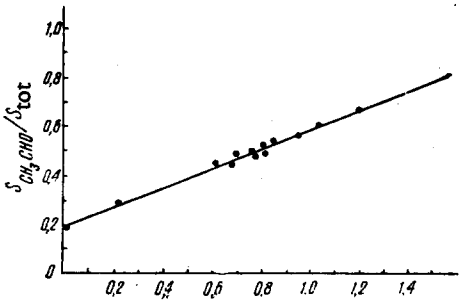

Fig. 2. Calibration graph for the analysis of the degree of substitution in hydroxyethylated partially hydrolyzed amylopectin starch.

In order to analyze the degree of substitution in hydroxyethylstarches a number of methods are used [3], in particular gas–liquid chromatography (GLC) [4]. This method is faster and simpler than chemical methods, although it requires the preliminary plotting of a calibration curve.

The present paper gives the results of a determination by the GLC method of the degree of substitution in hydroxyethyl derivatives of partially hydrolyzed amylopectin starch from waxy maize which we have obtained [5] and are being investigated as blood substitutes. Figure 1 shows chromatograms of the products of the pyrolysis of the hydroxyethyl derivatives of partially hydrolyzed amylopectin starch (1) and of the pyrolysis of the partially hydrolyzed amylopectin starch (2) that is the starting material for obtaining the hydroxyethyl derivatives. In both cases, on pyrolysis acetaldehyde is formed among the other products (its peaks on the chromatograms are hatched), but there is more of it in the products of the pyrolysis of the hydroxyethyl derivatives and, as Tai Han et al. have shown [4], its amount is proportional to the number of hydroxyethyl groups introduced into the starch molecule. In order to calculate the amount of acetaldehyde, in contrast to the procedure which they proposed, we used not the height of the peak on the chromatogram but the relative characteristic X, i.e., the ratio of the area of the acetaldehyde peak S_{CH_3CHO} to the total area of all the other peaks on the chromatogram, S_{tot}. The areas of the peaks were found by means of a planimeter. The calibration graph (Fig. 2) was plotted from the results of a determination of the degree of substitution in the hydroxyethyl derivatives of the partially hydrolyzed amylopectin starch by the chemical

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TABLE 1. Results of a Determination of the Degree of Substitution of a Number of Samples of Hydroxyethylated Partially Hydrolyzed Amylopectin Starch by the GLC and the Chemical Methods

Sample	Degree of substitution (chemical method)	GLC method		Δ degree of substitution
		$S_{CH_3CHO}/S_{tot}(X)$	degree of substitution	
I	0,96	0,55	0,96	0,00
II	0,77	0,50	0,81	0,04
III	1,01	0,58	1,03	0,02
IV	1,09	0,52	0,87	0,22
V	0,78	0,47	0,75	0,03
VI	0,70	0,44	0,66	0,04
VII	0,70	0,49	0,79	0,09
VIII	0,81	0,51	0,83	0,02
IX	0,88	0,53	0,89	0,01
X	0,83	0,50	0,81	0,02
XI	0,82	0,51	0,83	0,01
XII	0,81	0,50	0,81	0,00
XIII	0,22	0,29	0,25	0,03
XIV	0,61	0,44	0,65	0,04

method used for the analysis of the degree of substitution in hydroxyethylcellulose [6]. Since the products of the pyrolysis of the initial partially hydrolyzed amylopection starch also contained acetaldehyde, from its chromatogram it is possible to find the corresponding value of X, which is 0.19. Consequently, the beginning of the calibration graph does not pass through the origin but intersects the axis of ordinates above this point. This value is constant for a number of batches of partially hydrolyzed amylopection starch. The graph obtained (see Fig. 2) shows that the ratio of the areas of the peaks S_{CH_3CHO}/S_{tot} is directly proportional to the degree of substitution in the hydroxyethyl derivatives.

EXPERIMENTAL

The chromatographic analysis was performed in the following way: an accurately weighed sample of the hydroxyethyl derivative of partially hydrolyzed amylopectin starch (about 1 mg) was pyrolyzed in a sealed glass capillary (diameter 2 mm, length 50 mm) at 400°C for 10 min in a steel block with an electric heater. The capillary was placed in the evaporator of a Tsvet-4 chromatograph and it was diluted in the doser by means of a special device made in the manner recommended by Wendenburg [7]. The pyrolysis products passed into a column consisting of a copper tube with a diameter of 3.5 mm and a length of 2.4 m filled with Chromaton H (0.48-0.60 mesh) wetted with tricresyl phosphate. The liquid phase was taken in an amount of 15% of the weight of the solid phase. The temperature of the column was 45°C and that of the evaporator 200°C; flame-ionization detector. The carrier gas was helium and its pressure at the inlet to the column was 0.5 atm gauge.

It can be seen from Table 1 that all the samples of hydroxyethylated partially hydrolyzed amylopectin starch obtained by the hydroxyethylation of partially hydrolyzed amylopectin starch have a high degree of substitution. An exception is sample XIII (degree of substitution 0.22), which was isolated on hydroxyethylation with a ratio of the reactants (starch/ethylene oxide) different from all the other samples. For some samples of the hydroxyethyl derivatives (X, XIII, XIV) a check determination of the degree of substitution in hydroxyethylcellulose was made on a special apparatus (Vladimir Scientific-Research Institute of Synthetic Resins). The values found fit the calibration graph satisfactorily. We obtained similar results on using chromatographic columns with other solid and liquid phases: 5% of polyethyleneglycol on Polychrom (0.25-0.50 mm) and 15% on Celite-545. In order to make an accurate evaluation of the method that we used for analyzing the degree of substitution, we determined the square error of measurement X from the formula [8]

$$X = \pm \sqrt{\frac{\Sigma\alpha^2}{n-1}},$$

where α is the deviation of each measurement from the mean value of 10 determinations; n is the number of measurements.

The absolute value of the square error in the determination of X amounted to 0.045, which corresponds to a value of the square error in the analysis of the degree of substitution from the calibration graph of 0.08. In the chemical method, the error of the determination of the degree of substitution is 0.06.

The figures given in Table 1 show that the values of the degree of substitution obtained by the two methods agree well, and the differences in them (Δ degree of substitution) are less than the value of the square error in the majority of cases. In a comparative consideration of the results of the two methods of analysis of the degree of substitution in hydroxyethylated partially hydrolyzed starch it can be seen that the chemical method enables the degree of substitution to be determined with a somewhat greater accuracy, but the GLC

method is more convenient and simple, does not require special reagents, and enables the analysis to be performed more rapidly. It must be noted that there is little information in the literature on the chromatographic analysis of derivatives of biopolymers, and this imparts special interest to the work that we have performed.

SUMMARY

A gas–liquid chromatographic method has been used for analyzing the degree of substitution of a number of samples of hydroxyethylated partially hydrolyzed amylopection starch. A new method of plotting a calibration graph differing from that used previously is proposed.

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