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ACID HYDROLYSIS OF THE HYDROXYETHYL DERIVATIVE OF THE AMYLOPECTIN STARCH OF WAXY MAIZE

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Interest in starches and their chemical modifications has risen as the result of the appearance of reports on their successful use for medical purposes. According to the available literature information, hydroxyethylated starch (HES) is used for preparing a new blood substitute [1, 2], is being studied as a cryoprotector for erythrocytes [3-5] and other blood cells [6, 7], and is being used in leucophereses [8] and the preparation of erythrocytes [9]. To obtain HESs with different molecular weights, degrees of substitution, characteristic viscosities [η], and molecular-weight distribution, together with other methods acid hydrolysis, is used [2, 5], since the ether bonds in HESs are resistant to the action of acids and alkalis and the hydroxyethyl groups are not split off under the conditions of acid hydrolysis [10].

Continuing our investigations [11, 12] on the preparation and study of the physicochemical properties of hydroxyethyl derivatives of the amylopectin starch (HEAPS) of waxy maize [All-Union State Standard (GOST) 7697-66*], we have used acid hydrolysis to obtain a series of HEAPS hydrolysates with different physicochemical properties.

The present work was devoted to obtaining hydrolyzates of HEAPS with molecular weights of 200,000 (±20,000) and 50,000 (±5000) and a degree of substitution of 0.60-0.70, which may be of interest for their subsequent study as cryoprotectors, since there are positive results in the literature on the study as blood substitutes [2, 13, 14] and cryoprotectors [5, 15] of hydroxyethylated starches with molecular weights of 40,000-90,000 and 100,000-200,000 and degrees of substitution of 0.5-0.7. As the initial raw material for obtianing the HEAPS we used partially hydrolyzed amylopectin starch (PHAPS) with different degrees of polymerization [16].

TABLE 1. Results of the Determination of the Relative Viscosities of HEAPS during Hydrolysis

Expt. No.	Relative viscosity									
	Time of hydrolysis, min									
	0	10	20	30	40	50	60	70	80	90
1	5,9	—	2,9							
2	5,9	2,9	2,5							
3	5,8	2,9	2,7							
4	5,9	2,9	2,7							
5	5,9	2,9	2,5	2,3	—	1,9	1,8			
6	5,9	2,7	2,4	2,1	2,1	1,9	1,8			
7	5,7	2,9	2,5	2,1	—	1,9	1,8			
8	5,9	—	—	2,4	2,3	2,2	2,0			
9	5,7	2,9	—	2,3	2,1	1,9	1,9			
10	5,7	2,9	2,6	2,2	2,0	1,9	1,9			
11	11,4	3,5	2,9	2,5	2,3	2,1	2,0	1,9	1,8	1,7

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We performed 11 experiments on the acid hydrolysis of PHAPS in which the duration of hydrolysis was varied and the change in relative viscosity of an aqueous solution of the HEAPS was followed every 10 min.

It follows from Table 1 that the relative viscosities of the solutions change appreciably only in the first 10-20 min. The figures obtained show the good reproducibility of the results. We determined the molecular weights of freeze-dried hydrolyzates of HEAPS by the light-scattering method,* the degree of substitution by the GLC method [17], and by chemical analysis [18], the value of [η], and the specific rotation $[\alpha]_D^{20}$:

Hydrolysate	Number of the experiment	mol. wt.	Degree of substitution	[η], dl/g	$[\alpha]_D^{20}$, deg
HESH-1	1	$2,12\times10^5$	0,66	0,155	148,28±1,4*
	2	$1,87\times10^5$	0,66	—	—
	3	$1,98\times10^5$	0,69	-	—
	4	$2,09\times10^5$	0,70	0,152	147,4±1,4
HESH-2	1	$5,1\times10^4$	0,69	0,105	131,98±1,4*
	2	$5,0\times10^4$	0,66	0,102	132,47±1,4
	3	$5,1\times10^4$	0,70	—	—
	4	$5,2\times10^4$	0,69	0,102	132,85±1,4

*Means of three parallel determinations.

EXPERIMENTAL

The hydroxyethylation of the PHAPS was carried out by the method developed previously [11, 12], which consists in heating at 80°C for 45 min, with stirring, 1 liter of a 20% alkaline (pH 12.2) suspension of starch containing 42 g of freshly distilled ethylene oxide (GOST 7568-64, grade C). The reaction was performed in a stainless-steel autoclave with a jacket for thermostated heating.

The cooled solution was diluted twofold with distilled water and was neutralized with 6 N hydrochloric acid to pH 6.3, and the product was precipitated with acetone (2 liters), and was purified by two reprecipitations with acetone (4 liters). Traces of acetone were distilled off from an aqueous solution of HEAPS by distillation in vacuum from a rotary evaporator.

An aqueous solution of HEAPS with a concentration of about 5% was used for acid hydrolysis. Part of this solution was additionally purified by being boiled with activated carbon (2 g per liter of solution), filtration, and freeze-drying using the Usifroid (France) equipment for freeze-drying biopreparations at temperatures of −30 to 0°C (for 11 h 30 min) and of 0 to 20°C (for the period from 11 h 30 min to 15 h 30 min).

Using the gas–liquid chromatographic (GLC) method [17], for two purified HEAPS we determined the degree of substitution, which was 0.70, and also the relative viscosities of their 5% aqueous solutions, which were 12.3 and 6.4, respectively.

The acid hydrolysis of the HEAPS was carried out in a flask with a stirrer, thermometer, and reflux condenser. With stirring, 6 N hydrochloric acid was added dropwise to 1 liter of an aqueous solution of HEAPS with a concentration of about 5% until a normality of the reaction medium of 0.4 had been reached. Hydrolysis was performed at 70°C for 20, 60, and 90 min, the relative viscosity of the solution being determined every 10 min in an Ubbelohde viscometer at 20°C. The time of flow of distilled water was 103 sec (see Table 1). A solution of the hydrolysate was neutralized with 0.5 N caustic soda, filtered, and boiled for 10 min with activated carbon (2 g per liter of solution). After filtration, the HEAPS hydrolysates were isolated by precipitation with acetone taken in amounts of 1.5 and 2.5 liters per liter of solution for hydrolysates obtained at hydrolysis times of 20 min (HESH-1) and 60 min (HESH-2), respectively. After dissolution in distilled water and the elimination of the traces of acetone from the aqueous solution in vacuum in a rotary evaporator with heating in water bath, the hydrolysates were freeze-dried (see above).

SUMMARY

The conditions have been worked out for performing the acid hydrolysis of hydroxyethyl derivatives of the amylopectin starch of waxy maize.

*The molecular weights were determined by M. A. Mitsuk.

Hydrolysates of the hydroxyethyl derivative of the amylopectin starch of waxy maize have been obtained with molecular weights of 200,000 (±20,000) and 50,000 (±5000) which are recommended for further study as cryoprotectors. The hydrolysates were characterized by determination of the molecular weights, degrees of substitution, characteristic viscosity, and specific rotations.

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