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PRODUCTION OF GLUCOSE-FRUCTOSE SYRUPS FROM FERMENTATIVE HYDROLYSATES OF PLANT WASTES

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A method has been developed for obtaining glucose–fructose syrups by the hydrolysis of plant wastes under the action of a complex of cellulases from a hybrid strain of a fungus, followed by isomerization of part of the glucose to fructose by immobilized streptomycete glucose isomerase. The conditions for raising the degree of isomerization of the glucose and the period of half-inactivation of the immobilized glucose isomerase have been optimized, with a simultaneous cheapening of the process. Plant raw materials that have been used for obtaining glucose–fructose syrups are maize cobs, wheat or rice straw, and reeds. Isomerization has been achieved by streptomycete cells immobilized on cotton lint.

The organization of the industrial production of sugar substances and, in particular, sucrose and sucrose–fructose syrups (GFSs) is the most important problem of the food industry and of medicine, particularly for Uzbekistan. Great attention is being devoted to the use in biotechnological processes of specially selected strains of microorganisms and also their immobilized forms capable of the supersynthesis of enzymes [1-3].

Our task was to develop a method for obtaining GFSs from various cellulose-containing agricultural wastes with the aid of enzymes from microorganisms. The fermentative production of GFSs from plant wastes is more profitable and cheaper than acid hydrolysis.

A method of obtaining glucose syrups (GSs) with the aid of a cellulolytically active culture liquid of *Aspergillus terreus* has been described in detail previously [4]. The cotton lint used for the immobilization of glucose isomerase (GI) is a readily available and cheap waste of cotton production, and in Uzbekistan about 5 tonnes of it accumulates every day. For the isomerization of the GS a preparation of GI-based cotton lint and the cells of a highly active strain of *S..atratus* was used. The characteristics of the strain have been given in the literature [5, 6]. The method of obtaining GFSs included two stages: the production of a GS and the isomerization of some of the glucose of the GS to fructose. In the first stage, dry maize cobs or other plant wastes were ground in a mill to a particle size of 0.25-0.5 mm and were hydrolyzed with a complex of *Asp. terreus* cellulases. The hydrolysate — a glucose solution — was freed from unwanted salt components with the aid of activated carbon and ion-exchangers, after which its glucose content from various plant substrates amounted to (g/liter): reeds — 8.11; wheat straw — 9.2; rice straw — 9.35; maize cobs — 10. The highest yield of glucose was observed in the GS from maize cobs, and therefore this was used for isomerization after concentration by evaporation to 10-30%.

In the second stage, isomerization was effected with the aid of water-washed cells of a three-day culture of *S. atratus* immobilized on cotton lint. The methods of obtaining the immobilized preparations of glucose isomerase varied according to the sequence of modification of the substrates with a 16% solution of sodium citrate:

modified cotton lint was mixed with fresh streptomycete cells;

modified streptomycete cells were mixed with cotton lint;

immobilization of the glucose isomerase was achieved by aggregation within the modified cells.

In the first variant, during the period of half-inactivation 11.6 liters of GS containing 36 kg of glucose yielded 14.4 kg of fructose in the GFS. In the second variant, during the time of half-inactivation 4-5 liters of GS containing 1.5 kg of glucose was passed through a column. The yield of fructose in the GFS amounted to 1 kg. In the third variant, during the

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TABLE 1. Residual GI Activity after the Isomerization of a GS from Maize Cobs

Variant of cell immobilization	Residual GI activity (% of the initial) after the working of the column for (days)				
	5	10	15	20	25
1	100	86	80	55	45
2	75	50	20		
3	50	10			

time of half-inactivation 2.4 liters of GS containing 720 g of glucose was passed through the column. The yield of fructose in the GFS amounted to 200-300 g. In all the variants, 40% of the glucose was isomerized [sic].

Thus, with the same degree of isomerization of the glucose to fructose, the highest yield of fructose was observed in the first variant — using previously modified cotton lint for immobilization. The action of the preparation in the second variant — in which modified cells were mixed with cotton lint — was somewhat inferior, and the smallest amount of fructose was formed in the third variant — in which the enzyme was immobilized within the microbial cell.

It was established that in the continuous production of GFS containing 30% of glucose, under the optimum conditions it is possible to pass 11.6 liters of GS through the column during the period of half-inactivation (25 days) and to achieve the isomerization of 40% of the glucose to fructose. The residual activities of the immobilized glucose isomerase preparations varied according to their method of preparation (Table 1).

EXPERIMENTAL

Immobilized preparations of *S. atratus* were investigated. Various plant wastes were used as substrates for obtaining GFSs — maize cobs, wheat and rice straw, and reeds. A cellulolytically active culture liquid of *Asp. terreus* was used for hydrolysis, and cotton lint for immobilization.

Preparation of the Cotton Lint. Cotton lint was ground to a particle size of 1-2 cm and was mixed with a 16% solution of sodium citrate in a ratio of 1:25 (weight:volume), after which the mixture was left at room temperature for 10-12 h and was then filtered through a paper filter and was kept at room temperature for 5 days to give a moisture content of 12%.

Preparation of the Immobilized GI. The modified lint obtained was mixed with *S. atratus* cells in a ratio of 1:25 (by weight). The pH of the suspension was brought to 7.5 with sodium bicarbonate, and it was kept at 4°C for 12 h and dried at room temperature to a moisture content of 12%. The time of half-inactivation of the catalyst so obtained was 25 days. The activity did not fall below 50% during this period. The activity of the glucose isomerase was 250 units/g.

To obtain modified *S. atratus* cells, 2.5-3.5 g of cells of a three-day culture was stirred with 50 ml of a 16% solution of sodium citrate at 4°C for 12 h and the mixture was centrifuged. The modified cells so obtained were dried at room temperature for 5 days to a moisture content of 12%.

Isomerization Process. A thermostated column (1.5 × 30 cm) was filled with dry immobilized GI and the GSs from plant wastes, containing about 30% of glucose, were passed through it. The syrups were first alkalinized with 10% sodium bicarbonate to pH 7.8-8.0, and $MgSO_4 \cdot 7H_2O$ and $CoCl_2$ were added as cofactors in concentrations of 5×10^{-3} and 5×10^{-6} M, respectively.

The rate of pumping the syrup though the column was 20 ml/h at 70°C. The optimum time of the isomerization process was 580 h — the half-inactivation time of the GI. After this the residual activity of the GI amounted to 20%. During the period of half-inactivation, 11.6 liters of GS containing 36 kg of glucose was passed through the column. The yield of fructose in the GFS was 14.4 kg, i.e., 40% of the glucose had been isomerized.

The sugar levels in the GSs were determined by literature methods [7, 8], and the levels of fructose in the GFSs by the cysteine–carbazole method [9].

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