ENZYMATIC METHOD OF OBTAINING RUTIN

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The possibility has been shown for the first time of using enzymes for increasing the yield of rutin. The processes of fermentation and extraction have been optimized with the aid of mathematical methods of planning experimentation.

The main defect of the industrial technology for obtaining rutin is the incompleteness of its removal from the raw material in the extraction process, which is connected with the slow occurrence of the diffusion process. One of the methods of accelerating the diffusion process is the liberation of the molecules of the substance being extracted from the composition of the biopolymers of the plant cell. The preliminary comminution of the raw material, while substantially complicating the process of separating the extract and the meal, scarcely increases the yield of rutin.

We have studied the possibility of using enzymes with cellulytic activity in the extraction process. The results of preliminary experiments showed that with the aid of the enzymatic hydrolysis of the biopolymers of the plant raw material before extraction it is possible to achieve an increase in the extractability of flavonoids.

The influence of various parameters of the process on the yield of the final product was investigated by using the Box–Wilson method for the mathematical planning of experimental work [1]. The following main parameters affecting the process were selected: the temperature of fermentation – X_1; the concentration of enzymes – X_2; the time of fermentation – X_3; the pH of the solution – X_4; and the liquor ratio for extraction – X_5. In the optimization of the process we used a 1/4 replica from a complete factorial experiment with the application of planning of the 2^{5-2} type. We selected the levels of the factors and the intervals of their variation on the basis of the results of the investigations, and these are given in Table 1.

The experiments were carried out in accordance with the matrix of Table 2.

From the results of the experiments we found a regression equation with the following coefficients

$$Y = 65.4 + 0.68X_1 - 0.97X_2 + 2.14X_3 + 5.36X_4 + 0.19X_5 - 1.17X_1X_3 + 7.11X_2X_3.$$

To evaluate the significance of the regression coefficients we determined the dispersion of reproducibility from a well-known formula, $s_{repr} = 1.11$. The significance of each coefficient was evaluated by means of Student's criterion. As the calculations showed, all the coefficients were significantly apart from those for X_1 and X_5. Consequently, the results

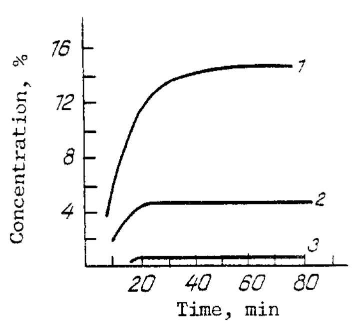

Fig. 1. Kinetic curves of the extraction of rutin: 1, 2, 3) concentrations of rutin in extracts I, II, and III, respectively (T = 130°C, liquor ratio 1:11).

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TABLE 1. Conditions for Planning an Experiment

Factor	Base level	Interval of variation	Lower level	Upper level	Unit of measurement
X_1	40,0	5,0	35,0	45,0	°C
X_2	0,35	0,15	0,2	0,5	%
X_3	45	25	30	60	min
X_4	3,0	1,5	1,5	4,5	pH
X_5	1:26	1:04	1:22		

TABLE 2. Planning Matrix and the Results of the Experiments

Expt. No.	X_1	X_2	X_3	X_4	X_5	Y_1	Y_2	Y_{av}	Y_{calc}	S^2
1	—	—	—	—	+	69,0	69,8	69,4	69,95	0,16
2	+	—	—	+	—	72,9	74,1	73,3	72,75	0,49
3	—	+	—	+	—	54,6	52,26	53,43	54.25	1,37
4	+	+	—	—	+	59,26	54.97	56,94	56,13	4,63
5	—	—	+	+	+	61,23	63,57	62,4	62,81	1,37
6	+	—	+	—	—	60,43	60,43	60,43	60,01	0
7	—	+	+	+	—	73,7	74,1	73,7	74,63	0,8
8	+	+	+	—	+	74.1	73,3	73,7	72,75	0,16

obtained in the realization of the matrix (Table 2) were near the optimum region. After the elimination of the nonsignificant coefficients, the regression equation assumed the form

$$Y=65.41-0.97X_2+2.14X_3+0.23X_4-1.17X_1X_3+7.11X_2X_3.$$

The equation obtained described the experiment adequately, as followed from the check by means of Fisher's criterion. Thus, the optimum conditions for performing the process of fermentation and extraction are the following: enzyme concentration 0.5% (on the weight of the raw material), fermentation time, 1.0 h, fermentation temperature 35°C, pH of the solution 4.5, liquor ratio 1:22.

The rate of extraction of the rutin was studied. The results of the investigation are given in Fig. 1. The kinetic curves of the process showed that in the first extraction an equilibrium of phases set in after 1 h 20 min, and the concentration of the extract was then 15%; in the second extract the corresponding values were 40-50 min and a concentration of 5%; and the third extract at a liquor ratio of 1:11 contained ~0.3% of rutin, from which it was subsequently impossible to isolate any amount whatever of the product. It follows from this that the performance of three extractions under industrial conditions is undesirable, i.e., two extractions with a total time of 2 h, including the process of enzymatic hydrolysis, are sufficient.

EXPERIMENTAL

Fermentation of the Raw Material. With stirring for 15 min, 0.5 g of the enzyme was dissolved in 1100 ml of universal buffer solution with pH 4.5. The solution so obtained was poured on to 100 g of Japanese pagoda tree flower buds and the mixture was incubated at 35°C for 1.0 h.

Extraction. After enzymatic hydrolysis, extraction with water was carried out at 130°C with stirring for 1.0 h. The extract obtained was separated from the meal and was cooled. The residual meal was extracted again under the same conditions with a shortening of the time of extraction to 50 min. The extracts obtained were combined and cooled to 18-19°C. A greenish-yellow precipitate of technical rutin then deposited. The precipitate that had deposited was filtered off, dried at 80°C, and recrystallized from alcohol.

LITERATURE CITED

1. S. L. Akhnazaroba and V. V. Kafarov, The Optimization of Experiments in Chemistry and Chemical Technology [in Russian], Moscow (1978), p. 99.