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OPTIMIZATION OF THE PROCESS OF EXTRACTING AMYLOLYTIC ENZYMES FROM THE TECHNICAL PRODUCT "AMILORIZIN-P_x"

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UDC 615.35.015.4:612.3

In the process of obtaining the medicinal enzyme preparation "Oraz," which has been approved for use in medical practice as an agent for the treatment of diseases of the gastrointestinal tract, one of the main steps is the aqueous extraction of the enzymes from the initial raw material "Amilorizin-P_x," which is a culture of the mold fungus Aspergillus oryzae surface-grown on wheat bran [OST (All-Union Standard) 59-6-72].

As the method of extracting the active substances from this raw material we selected extraction in a battery of percolators by the countercurrent principle which, in the opinion of a number of authors [1-3], ensures the most complete extraction of the active components both from plant medicinal raw material and from surface cultures of mold fungi.

In view of the fact that this process is a complex one and depends on various factors, it appeared desirable to carry out its optimization.

To solve this problem we used the method of mathematical planning of experiments with the aid of Latin squares [4, 5]. The process of extracting the amylolytic enzyme was studied as a function of the following factors, which, according to the literature and preliminary experimental results, play a fundamental part in it: A – the selected volume of the extract, ml; B – the number of percolators in a battery; and C – the time of steeping, min. The levels of the factors mentioned are given below:

Factor	Level of the factor			
A	200	400	600	800
B	2	3	4	6
C	10	15	20	25

As constant factors of the extraction process we took the temperature of extraction (20-25°C), the method of extraction (countercurrent), the sequence of technological operations in the subsequent isolation of the "Oraz" preparation, and also the nature of the extractant (water).

For planning the experiment we used a 4 × 4 Greco-Latin square according to a method described in the literature [5].

The optimization parameters were the saccharifying power of the "Oraz" preparation and its yield calculated to unit weight of the initial raw material, the values of which, according to a Provisional Pharmaceutical Article (VFS-42-570-76) should be not less than 200 units per gram of preparation and 1%, respectively.

The matrix for the planning of the experiment is given below (the results of the investigations are given in Table 1):

Kharkov Scientific-Research Institute of Pharmaceutical Chemistry. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 62-66, January-February, 1979. Original article submitted May 25, 1978.

0009-3130/79/1501-0051$07.50

TABLE 1. Results of Experiments and Preliminary Treatment of these Results

X_1	X_2													
	B_1		B_2		B_3		B_4		A_{tot}		Mean totals		Squares of the totals	
	SA, units/g	yields, %	SA, units/g	yields, %	SA, units/g	yields, %	SA, units/g	yields, %	SA, units/g	yields, %	SA, units/g	yields, %	SA, units/g	yields, %
A_1	337,0	1,9	266,2	3,2	254,3	5,4	254,3	5,2	1111,8	15,8	277,9	3,9	1236099,2	250,9
A_2	299,8	0 8	299,8	0,9	299,8	1,1	254.3	1,5	1153,7	4,5	288,4	1,1	1331023,6	20,2
A_3	266,2	0,3	299,8	0,4	312,5	0,4	266.2	0,4	1144,7	1,7	286,1	0,4	1310338,0	2,9
A_4	266,2	0,1	277,0	0,2	312,5	0,2	254,3	0,3	1110,0	1,0	277,5	0,2	1232100,0	1,0
B_{total}	1169,2	3,3	1142,8	4,8	1179,1	7,2	1029,1	7,6						
Mean totals	292,3	0,8	285,7	1,2	294,7	1.8	257,2	1,4						
Squares of the totals	1367028,6	10,9	1305991,8	23,9	1390276,8	52,8	1059046,8	57,9						

Effect of item C	C_1		C_2		C_3		C_4	
	SA, units/g	yield, %	SA, units/g	yield, %	SA, units/g	yield, %	SA, units/g	yield, %
B_{total}	1180,0	3,8	1132,8	6,8	1099,2	5,3	1108,2	7,0
Mean totals	295.0	0,9	283,2	1,7	274,8	1,3	277.0	1,7
Squares of the totals	1392400.0	14,6	1283235,8	46,5	1208240,6	28,9	1228107,2	49,7

TABLE 2. Dispersion Analysis of the Experimental Results

Source of the dispersion	No. of degrees of freedom	Sum of the squares		Mean square		F-experimental		F-tabular	
		A, units/g	yield, %	A, units/g	yield, %	A, units/g	yield, %	A, units/g	yield, %
Rows	3	377,2	35,5	125,7	11,8	0,14	18,77	4,76	4,76
Columns	3	3573.0	3,1	1191,0	1.0	1,34	1,63	4,76	4,76
Item C	3	982,9	1,6	327,6	0,5	0,37	0,87	4.76	4,76
Errors	6	5308,1	3,8	884,4	0,6				
Total	15	10241,3	44,1						

Levels of the factors (A, B, C)

	B_1	B_2	B_3	B_4
A_1	C_1	C_3	C_4	C_2
A_2	C_4	C_2	C_1	C_3
A_3	C_2	C_4	C_2	C_1
A_4	C_3	C_1	C_3	C_4

It can be seen from the results of a dispersion analysis that are given in Table 2, that the linear effects of the factors investigated on the saccharifying activity of the preparation (SA) proved to be insignificant, since in all cases the value of Fisher's F criterion F-experimental was lower than F-tabular.

Of the linear effects of the factors investigated on the yield of the preparation, only the effect characterizing the volume of extract taken from the percolator (factor A) proved to be significant. The other factors proved to be less important under the conditions selected.

By comparing the mean values for each factor separately it is possible to determine the optimum conditions for the extraction process according to which the activity and yield of the preparation will also have the optimum values. The levels of these conditions with respect to the SA were $A_2B_3C_1$, and with respect to the yield of preparation $A_1B_3C_4$.

In view of the fact that an increase in the number of percolators in the battery leads to a rise in the resistance to the flow of extractant, and also to a fall in the efficiency of the use of the industrial area, it appeared desirable, from technological considerations, to test not only the optimum conditions found, $A_2B_3C_1$ and $A_1B_3C_4$, but also the conditions $A_1B_2C_3$, according to which three percolators are used in the battery. This led to only a slight increase in activity and to a small decrease in the yield of preparation. The results of the test are given below:

Conditions of the extraction process tested	SA of the preparation, units/g	Yield of preparation, %
$A_1B_3C_4$	243.5	4.87
$A_2B_3C_1$	299.8	1.01
$A_1B_2C_3$	266.2	3.19

As can be seen from these figures the optimum conditions of extraction are represented by $A_1B_3C_4$, i.e., the volume of extract taken from each percolator should be 200 ml, the number of percolators in a battery 4, and the time of steeping of a percolator 25 min. However, the results corresponding to extraction conditions $A_1B_2C_3$ are close and lead to a slight increase in the activity of the preparation together with a slight fall in its yield.

EXPERIMENTAL

In each individual case, to perform extraction a battery of percolators was set up on the countercurrent principle in accordance with the plan of the experiment (see below). Each percolator was charged with 200 g of the raw material "Amilorizin-P_x" (OST 59-6-72). Mains water was added from an overhead tank until the percolator was filled, and steeping was carried out in accordance with the values of factor C. Then the battery was started up. The first "regime" outflow of extract was used for the subsequent isolation of the "Oraz" preparation by the scheme adopted [6].

The activity of the preparation was determined by a standard method [7]. The yield of the preparation was calculated to unit weight of the initial raw material. The statistical treatment of the results of the experiment was performed as described in the literature [5].

SUMMARY

1. The influence of the main factors on the process of extracting amylolytic enzymes from the raw material "Amilorizin-P_x" has been studied.

2. The optimum conditions for performing the extraction, leading to a preparation corresponding to the requirements of the appropriate Provisional Pharmacopoeial Article have been selected.

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