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# A STUDY OF THE OPTIMUM CONDITIONS FOR THE EXTRACTION OF UREASE FROM THE SEEDS OF THE WATERMELON *Citrullus vulgaris*

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The influence of a number of factors on the extraction of urease from the seeds of the watermelon *Citrullus vulgaris* Schrad. has been investigated by the method of mathematical planning of experimental work. On the basis of the results obtained, using the method of steepest ascent, the optimum conditions of the extraction process ensuring the maximum specific activity of the enzyme have been determined.

Recently, enzyme preparations have been finding ever greater use in medicine [1, 2]. In addition to the main direction of the use of enzyme preparations in substitution therapy, to supplement a deficiency of enzymes causing various diseases, there is interest in the use of enzymes for diagnosis and as catalysts of the hydrolysis of particular toxic products of the metabolism. One such enzyme is urease, which catalyzes the hydrolysis of urea. An analysis of the literature shows a great interest in the use of urease for the purposes of diagnosis [3-5] and, in the immobilized form, for the hydrolysis of urea in "artificial kidney" apparatuses [6].

The most important stage in the isolation of urease from plant raw material is the extraction process. The process of extracting enzymes is affected by such factors as the degree of comminution of the raw material, the temperature of the process, the time of extraction, the pH of the extractant, and the ratio of raw material and extractant. To check the significance of these factors we have performed an experiment of the $2^{5-2}$ fractional replica type. The use of such a fractional replica permits the determination of the significance of a large number of factors in the determination of optimum conditions with the minimum number of experiments and enables the time of experimentation to be substantially shortened [7, 8].

To perform the experimental investigations we introduced the following coding of the factors:

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TABLE 1. Plan and Results of the experiment

| Number | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $Y_e$ | $Y_c$ | $Y_e - Y_c$ | $(Y_e - Y_c)^2$ |
|---|---|---|---|---|---|---|---|---|---|
| 1 | − | − | − | + | − | 273,0 | 273,05 | | |
| 2 | − | + | − | − | + | 146,5 | 138,59 | | |
| 3 | + | − | − | − | + | 117,8 | 125,63 | | |
| 4 | + | + | − | + | − | 186,2 | 186,05 | | |
| 5 | − | − | + | + | + | 162,0 | 161,87 | | |
| 6 | − | + | + | − | − | 180,2 | 188,05 | | |
| 7 | + | − | + | − | − | 183,0 | 175,09 | | |
| 8 | + | + | + | + | + | 74,8 | 74,87 | | |
| 9 | 0 | 0 | 0 | 0 | 0 | 165,9 | 165,25 | +0,65 | 0,4225 |
| 10 | 0 | 0 | 0 | 0 | 0 | 165,3 | 165,25 | +0,05 | 0,0025 |
| 11 | 0 | 0 | 0 | 0 | 0 | 164,2 | 165,25 | −1,05 | 1,1025 |
| 12 | 0 | 0 | 0 | 0 | 0 | 165,1 | 165,25 | −0,15 | 0,0225 |
| 13 | 0 | 0 | 0 | 0 | 0 | 165,7 | 165,25 | +0,45 | 0,2025 |
| 14 | 0 | 0 | 0 | 0 | 0 | 165,3 | 165,25 | +0,05 | 0,0025 |

$\Sigma = 1,7550$

| Factor | Particle size, nm | pH of the extractant | Time of extraction, h | Temperature of the process, °C | Ratio of raw material and extractant |
|---|---|---|---|---|---|
| Code symbol | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ |
| Base level $X_0$ | 3 | 7,0 | 2 | 20 | 1:5 |
| Interval of variation, $\Delta X$ | 2 | 1,0 | 1 | 10 | 1:2 |
| Upper level, +1 | 5 | 8,0 | 3 | 30 | 1:7 |
| Lower level, −1 | 1 | 6,0 | 1 | 10 | 1:3 |

The decisive contrasts of the given quarter-replica are as follows: $X_4 = X_1 \cdot X_2$, and $X_5 = X_1 \cdot X_2 \cdot X_3$.

The optimization parameters used were the specific activity of the urease, expressed in Sumner units referred to unit of protein.

On the basis of the values of $Y_e$ obtained, for each experiment we determined the coefficients of the regression equation:

$$B_0 = \frac{\Sigma_Y}{n} = \frac{1323.5}{8} = 165.4, \quad B_1 = \frac{\Sigma_Y \cdot X_1}{n} = -24.99,$$

$$B_2 = -18.51, \quad B_3 = -15,43, \quad B_4 = +8.56, \quad B_5 = -40.16.$$

The calculation of the estimate of the dispersion of an experiment was carried out on the basis of the results obtained in the center of the plan with respect to the base level of all the factors (experiments 9-14).

$$Y_p = \frac{\Sigma_{Y_0}}{n} = 165.25, \quad S_Y^2 = 1.7550 : 5 = 0.351, \quad S_{Bi}^2 = 0.351 : 8 = 0.044, \quad S_{Bi} = \sqrt{0,044} = 0.21.$$

On calculating the values of the t ratios (Student's criterion) and comparing them with the tabular value $0.95^{(5)} = 2.57$, we convinced ourselves that the experimental values of the t ratios for all the coefficients were greater than the tabular values and, consequently, all the coefficients were significant:

$$t_0 = \frac{B_0}{S_{Bi}} = \frac{165.4}{0.21} = 787.6, \quad t_1 = 119.0, \quad t_2 = 88.14, \quad t_3 = 73.47,$$

$$t_4 = 40.76, \quad t_5 = 191.24.$$

On the basis of the results obtained, the process of the extraction of ureas from watermelon seeds can be described by the following equation:

$$Y = 165.4 - 24.99X_1 - 18.51X_2 - 15.43X_3 + 8.56X_4 - 40.16X_5.$$

Let us determine the adequacy of the equation to the experimental results. For this purpose, by substituting in the equation the values of Y for each factor we obtain $Y_c$. Since the experimental and calculated values of Y do not coincide at each point of the plan, the theoretical deviations from the experimental values, the squares of the deviations, the dispersion of adequacy, and the experimental value of the Fisher criterion are considered. The value obtained $F_e = 0.13$ is compared with the tabular value $F_{0.95}(2.8) = 4.46$, and since $F_e < F_{tab}$ all the coefficients of the equation are adequate to the results of the experiment.

In an analysis of the signs of the coefficients of the regression equation it is possible to draw the conclusion that the yield of the process (specific activity) rises with a decrease in the degree of comminution of the raw material ($X_1$), in the pH of the attractant ($X_2$), in the type of extraction ($X_3$), and in the ratio of raw material and extractant ($X_5$) and with a rise in the temperature of the process ($X_4$). The coefficients of the equations enable us to judge the degree of influence of each factor. It is obvious that the greatest influence is exerted by the ratio of raw material and extractant ($B_5 = -40.16$), and then the comminution of the raw material ($B_1 = -24.99$) and, to a somewhat smaller degree, the pH of the extractant ($B_2 = -18.51$) and the time of extraction ($B_3 = -15.43$). The temperature of the process has only a slight effect ($B_4 = +8.56$).

The finding of the coefficients of the regression equation enabled the extraction process to be optimized. For this purpose we used the method of steepest ascent:

| Characteristic | | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $Y_0$ |
|---|---|---|---|---|---|---|---|
| Base level, $X_0$ | | 3.0 | 7.0 | 2 | 20 | 1:5 | |
| Interval of variation $\Delta X$ | | 2.0 | 1.0 | 1 | 10 | 1:2 | |
| Coefficient Bi | | −24.99 | −18.51 | −15.43 | +8.56 | −40.16 | |
| $\Delta X \cdot Bi$ | | −49.98 | −18.51 | −15.43 | +85.6 | 80.32 | |
| Calculation step h | | −0.5 | −0.18 | −0.15 | +0.85 | −0.8 | |
| Rounded-off step h | | −0.5 | −0.2 | −0.15 | +1.0 | −0.8 | |
| Imaginary experiments | 1 | 3.0 | 6.8 | 1.85 | 21 | 1:4.2 | 274.82 |
| | 2 | 2.5 | 6.6 | 1.70 | 22 | 1:3.4 | 283.33 |
| | 3 | 2.0 | 6.4 | 1.55 | 23 | 1:2.6 | 211.95 |
| | 4 | 1.5 | 6.2 | 1.40 | 24 | 1:1.8 | 178.22 |
| | 5 | 1.0 | 6.0 | 1.25 | 25 | 1:1 | — |
| | 6 | 0.5 | 5.8 | 1.1 | 26 | 1:0.2 | — |

As the unit step we took a change in the particle size of the raw material by 0.5 mm. For the other factors, the size of the step was decreased by a factor $\frac{\Delta X \cdot Bi}{0.5} \approx 100$. The conditions of experiment 2 are taken as optimal. Thus, to achieve the maximum yield of the extraction process (specific activity of the enzyme) the following are required: raw material with a particle size of 2.5 mm, extractant with a pH of 6.6, time of extraction 1.70 h (1 h 42 min), temperature of the process 22°C, and ratio of raw material and extractant 1:3.4.

From technological considerations, in the process of obtaining urease it is possible to carry out extraction within the limits of the experiments 1 and 2 for all the factors of the regression equation. Experiments 5 and 6 were not performed, since at a ratio of 1:1 the extractant was absorbed by the raw material with the required particle size.

## EXPERIMENTAL

In the performance of the experiments we used seeds of the table watermelon of the Ogonek variety, species Citrullus vulgaris Schrad. The seeds were comminuted by a single method – crushing with a smooth-roll crusher. For the sieving analysis of the comminuted plant raw material we used a sieve according to GOST [All-Union State Standard] 214-57. The extractant was 0.1 M sodium-potassium phosphate buffer. The pH of the extractant was checked on a model pH-340 pH meter. A weighed sample of raw material with the given degree of comminution was extracted by the maceration method. The meal was pressed through gauze and the final clarification of the extract was achieved on a TsLR-refrigerated centrifuge at 4°C and a speed of 3000 rpm for 25-30 min. Urease activity was determined by Sumner's method [9], and the concentration of protein in the extract by Lowry's method [10]. The optical densities of colored solutions were measured on a FÉK-56M photoelectric colorimeter in cells with a layer thickness of the liquid of 10 mm using light filters 5 and 8, respectively, for the above-mentioned methods.

SUMMARY

The influence of the main factors on the process of extracting urease from water melon seeds has been studied. The optimum values of the factors, degree of comminution, pH of the extractant, time of extraction, temperature of the process, and ratio of raw material and extractant have been determined.

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# AN INVESTIGATION OF THE FRACTIONAL COMPOSITION OF DIOXANE LIGNIN OF THE COTTON PLANT BY THE PMR METHOD

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Analysis of the PMR spectra of six fractions of the dioxane lignins of the cotton plant differing considerably in molecular weight has confirmed their chemical nonidentity. It has been established that the low-molecular-weight fractions are less condensed than the high-molecular-weight fractions. It has been found that aliphatic OH groups are approximately evenly distributed between the $\alpha$- and $\gamma$-C atoms of the lignin side chain.

We have separated the dioxane lignin of the ripe stems of the cotton plant of variety 108-F (DLA) into six fractions differing considerably in molecular weight [1]. The chemical nonidentity of the fractions has been shown by the fact that they contain different amounts of functional groups in the phenylpropane structural units (PPSUs), by a comparison of the relative optical densities of the main bands in their IR spectra, and also by an analysis of the products of alkaline nitrobenzene oxidation [2]. Continuing a study of the fractions obtained, we have investigated their PMR spectra after acetylation. As was shown previously, this method gives extremely valuable information in the comparative study of different dioxane lignins of the cotton plant.

The conditions of recording the PMR spectra and the methods for treating them have been given previously [3]. The results of the present investigation are given in Table 1.

Analysis of the numbers of free aromatic protons (zone I) showed that in the various fractions they ranged from 2.02 to 2.16 per $C_9$. Knowing that in all fractions the amount of p-coumaryl structures was 10-15 times

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