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RESEARCH ARTICLE

Mathematical Design of the Experiment in the Development of Technology of Beeswax Obtaining from Propolis

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Abstract

In present material the development of the technology of purified beeswax obtaining from propolis, which meets modern physical-chemical and therapeutic requirements, using a various models of mathematical design of the experiment has been shown. In this work a petroleum-ether fraction of propolis containing a beeswax product was used. While analyzing of the obtained fraction, lipids and some other high-molecular compounds of unqualified structure are extracted in parallel, as well as an insignificant number of the γ-pyrone derivatives. In this connection, the development of the condition of beeswax obtaining from this fraction, which is maximally purified from the related substances, was reasonable. For this purpose a series of classical methods of fractionally differentiated extraction followed by fractions evaporation in a vacuum was used. In addition, methods of precipitation using a some organic solvents were used. The optimal conditions of the technology of purified beeswax obtaining from propolis have been determined by methods of mathematical design.

Keywords: *Propolis, Beeswax, Technology, Methods of fractionally differentiated extraction, Precipitation, Methods of mathematical design.*

Introduction

At present, one of the main tasks of pharmaceutical science is the creation of highly effective drugs for the prevention and treatment of various diseases. The solution of this problem is possible in the presence of a substantial arsenal of medicines in various dosage forms. However, the development of modern medicines requires the using of auxiliary substances, in particular excipients, preservatives, stabilizers, prolongators, bases, etc. [1].

In pharmaceutical technology a significant part of substances listed in the State Register of Medicines and authorized for medical usage and for industrial production are used. [2] Among the range of auxiliary substances with the properties to create a certain consistency, viscosity, plasticity and ability of the dosage form to show directed pharmacological activity, beeswax has a certain value [3-5].

At the same time, his needs in the medical industry, pharmacy production of medicines and other branches of the national economy are satisfied only by 50-60 % currently. Especially, it is acutely felt in pharmacy, cosmetic, paint and metallurgy industries [6].

However, despite tremendous progress in the field of synthesis and chemical technology, wax, similar to created by bees in a hive and meeting modern requirements, has not been achieved yet. In this connection, the search for economically rational and available raw materials for obtaining beeswax has proved the expediency of using propolis, one of the beekeeping products, containing up to 30 % of beeswax and considered as a waste in the production of finished medicines based on it [7, 8].

In the aspect of the above, the purpose of this work was to develop the technology of purified wax obtaining from propolis, which meets modern physical-chemical and therapeutic requirements, using a various models of mathematical design of the experiment.

The realization of this goal required a solving of following tasks for development of the technology of beeswax extracting in the purified state, namely:

- To determine the conditions for evaporation of petroleum-ether extraction;
- To establish the required amount of extract;
- To develop the optimal conditions for quantitative and qualitative precipitation of wax products;
- To conduct a physical-chemical researches of propolis wax.

Materials and Methods

In this work, a petroleum-ether fraction of propolis containing a wax product was used. While analyzing of the obtained fraction, lipids and some other high-molecular compounds of unqualified structure are extracted in parallel, as well as an insignificant number of the γ-pyrone derivatives. In this connection, it was expedient to develop the conditions of beeswax obtaining from this fraction, which is maximally purified from the related substances. For this purpose a series of classical methods of fractionally differentiated extraction followed by fractions evaporation in a vacuum was used.

In addition, methods of precipitation using a some organic solvents were used. As a result of complex researches, the most optimal conditions of purified wax obtaining from petroleum-ether extract of propolis is the following technology: propolis-raw was crushed, sifted through a sieve No. 05, placed in an extractor, poured with petroleum ether to a mirror surface in a ratio 1:5 and was heated on the water bath under reflux condenser at the temperature not higher than 60 ° C about 50-60 minutes.

The obtained extracts were evaporated to 200-300 ml of volume, transferred quantitatively to 3000 ml container and added to this extract 8-10 times of ethanol 95 % cooled to -5-7 ° C.

The intense white precipitation was carefully filtered in vacuum, dried until the solvent was completely removed, and transferred to a storage container. For the purpose to optimize of technological processes and to prevent unnecessary costs while production, mathematical methods of experiment design were used making it more efficient and economical. At present, there are several approaches to the mathematical study of unknown phenomena in scientific researches while technological processes of the natural raw materials processing [9-14].

On the one hand, a simplified model is taken as the basis of the experiment, using differential, integral or other analytical dependences, which can be confirmed with a certain degree of approximation of the research results. On the other - on the basis of the carried out analysis, the corresponding models are constructed, according to results of statistical processing obtained by sifting "insignificant" factors that were taken into account in the course of our experimental design.

The application of methods of active experiment makes it possible to obtain the mathematical models describing the properties of objects of the research, while is no need to evaluate the processes occurring within the object. The obtaining of mathematical model is ensured by the precise implementation of the algorithm of researches and the determination of the values of the object response function [15, 16].

The design of the experiment makes it possible to vary all factors simultaneously and to obtain quantitative estimates of both the main factors and the interaction effects between them, and the results obtained are characterized by a smaller error than the traditional methods of one-factor study [17, 18].

Taking into account the strong practical interest to beekeeping products, in particular to beeswax, the development of the optimal technology for their extraction from natural sources is an actual. In this connection, in order to optimize the technological process of purified beeswax obtaining from propolis in the experiment we also used methods of mathematical design.

At larger number of independent technological factors, it was necessary to search for that area of the factor space in which the extraction of beeswax would be the most maximum.

Regression regularities obtained during the design of the experiment were used to solve the task. In this connection, in order to optimize the environment, in our opinion, the method of factor experiment is expedient, which provides a large number of experiments, realize possible combinations of the basic levels of independent variable factors, establish their optimal levels, and also to find and justify the optimal technological parameters faster than the empirical method.

Results and Discussion

Based on the results of conducted experiment for optimization of the extracting process of beeswax from propolis using the steepest ascent method, which combines the design of the experiment with the gradient marching method [19], has been experimentally established that six factors influence on the beeswax content (Y), namely: quantitative ratio of propolis and solvent (X_1); time of single extraction, min (X_2); number of repetitions (multiplicity of extractions) (X_3); degree of raw materials extraction, mm (X_4); temperature, °C (X_5); number of stirring while the single extraction (X_6). The results are shown in Table 1.

Table 1: Experimental data of beeswax extraction from propolis

No of sample	X_1 (parts)	X_2 (min)	X_3 (number)	X_4 (mm)	X_5 (°C)	X_6 (number)	Y (%)
1	1 : 5	60	5	2	80	4	30
2	1 : 10	50	4	5	40	7	20
3	1 : 15	40	4	2	50	6	25
4	1 : 1	60	6	4	70	2	35
5	1 : 10	45	5	3	30	8	15
6	1 : 5	50	3	3	70	3	30
7	1 : 15	35	5	5	50	5	25
8	1 : 1	50	6	2	60	4	27
9	1 : 20	30	4	5	50	5	30
10	1 : 25	20	2	4	60	4	20
11	1 : 20	25	3	3	60	3	23
12	1 : 5	30	6	4	40	6	10
13	1 : 2	60	7	3	70	10	32
14	1 : 4	50	6	4	60	7	25
15	1 : 10	40	5	5	50	9	20
16	1 : 30	30	4	2	50	6	15

Notes*: X_1 – quantitative ratio of propolis and solvent; X_2 – time of single extraction, min; X_3 – number of repetitions (multiplicity of extractions); X_4 – degree of raw materials extraction, mm; X_5 – temperature, °C; X_6 – number of stirring while the single extraction; Y – beeswax content, %.

The process of statistical processing of the results of experimental researchers was divided into two stages:

- A study of the relationship between the percentage of beeswax extracted from propolis (dependent variable Y) and independent variables (X_1, X_2, X_3, X_4, X_5, X_6) (Table 1) with the determination of the correspondence of the selected linear model to the experimental results;
- Search of the optimal conditions for beeswax extraction from propolis and purification.

In the course of the experiment, the parameters X_1-X_6 were checked for the purpose to determine the most significant ones among them.

For the construct of the mathematical model, a factorial experiment was performed, where the quantitative content of beeswax (Y, %) was as a parameter of optimization (dependent factor), and values of the parameters X_1 – X_6 were as an independent factors. While designing according to this scheme, the upper and lower levels were established experimentally. Based on the average values of the parameters X_1 – X_6, the center of the plan and the variability step were determined. The verification showed that the experimental data are normally distributed and homogeneous. For the construction and analyzing of empirical models in our experiment, the following criteria were used: the choice and optimization of the model, as well as the evaluation of the obtained results. The calculation for choosing of model was carried out according to the formula of linear dependence, which is the hyperplane equation in the space of *n*-parameters of independent x_j.

$$y = b_0 + \sum_{j=1}^{m} b_j x_j \quad (1)$$

Where: b_j – coefficients for unknowns;
b_0 – absolute term.

For selection of the system parameters b_0, b_j, the linear estimation as well as regressive analysis were used to optimize the model [20]. These parameters were found by the least square method (LS method), based on minimizing the sum of the squared deviations of the observed values of y_j from their mathematical expectations $\bar{y}_j$, that is:

$$\sum_{i=1}^{n} (y_j - \bar{y}_j)^2 \rightarrow min \quad (2)$$

The regression coefficients were calculated as follows:

$$b_j = \beta_j \frac{S_y}{S_j} \quad (3)$$

Where: S_y - standard deviation of the dependent variable; S_j - standard deviation of the independent variable. $j = 1, 2, \ldots m$

$$\beta_j = \sum_{j=1}^{m} r_{iy} \cdot r_{iy}^{-1} \quad (4)$$

Where: r_{iy}– inters correlation of the *i-th* independent variable with the dependent variable; r_{iy}^{-1} – inverse value of intercorrelation r_{iy}. The standard deviation S_j was determined by formula:

$$S_j = \frac{\sqrt{S_{jk}}}{\sqrt{N-1}} \quad (5)$$

And the correlation coefficient was calculated by following formula:

$$r_{iy} = \frac{1}{N} \sum_{n=0}^{N-1} x_i(n) y(n) \quad (6)$$

Where: N – a number of observations; S_{jk} – a covariance.

$$S_{jk} = \sum_{i=1}^{N} (x_{ij} - \bar{x}_j)(x_{ik} - x_k) \quad (7)$$

Where: $\bar{x}_j$– a middle value.

$$\bar{x}_j = \frac{\sum_{i-1}^{N} x_{ij}}{N} \quad (8)$$

Where: x_{ij} is the independent variables;
$i = 1, 2 \ldots N$ – observations;
$j = 1, 2 \ldots m$ – variables.

$$b_0 = \bar{y} - \sum_{j=1}^{m} b_j \cdot x_j \quad (9)$$

Where: $\bar{y}$ is a middle value of the dependent variable? The model was evaluated by two ways:

- Within the regression line, based on the study of variance of the regression coefficients;
- Regression lines in general, that is, on the adequacy of this model.

The square of the multiple correlation coefficients for R^2 serves as a measure of optimizing the empirical model as a whole, which was determined as follows:

$$R^2 = \sum_{j=1}^{m} \beta_i \cdot r_{iy} \quad (10)$$

The sum of deviations square from regression was found by formula:

$$SS = D_{yy} - R^2 \cdot D_{yy} \quad (11)$$

Where: D_{yy} is the sum of deviations square from the middle value for the dependent variable.

The value of the Fisher's ratio test was determined by the formula:

$$F = \frac{R^2 \cdot D_{yy}(n - m - 1)}{(D_{yy} - R^2 \cdot S_{yy})m} \quad (12)$$

The dispersion was determined from equation:

$$S_{\bar{y}}^2 = \frac{SS}{n - m - 1} \quad (13)$$

The standard error was calculated as follows:

$$S_y = \sqrt{S_{\bar{y}}^2} \quad (14)$$

The standard deviations of the regression coefficients were found from the formula:

$$S_{bj} = \sqrt{\frac{r_{iy}{}^{-1} S_{\bar{y}}^2}{D_{yy}}} \quad (15)$$

Where: D_{yy} is the sum of deviations square from the middle value.For the independent variable. Student t-test was determined by the formula:

$$t_j = \frac{b_j}{S_{bj}} \quad (16)$$

These calculations were carried out according to results of experimental data using the Statistica 10 Stat Soft Inc system [21-24], on the basis of which the regression equation was obtained for the extraction process of wax products from propolis (Fig. 1).

Regr. Coefficients; Var.:Y; R-sqr=,74686; Adj:,5781 (Extraction of Vax.sta)

Factor	Regr. Coefficients; Var.:Y; R-sqr=,74686; Adj:,5781 (Extraction of Vax.sta) 6 factors, 1 Blocks, 16 Runs; MS Residual=20,01917 DV: Y					
	Regressn Coeff.	Std.Err.	t(9)	p	-80,% Cnf.Limt	+80,% Cnf.Limt
Mean/Interc.	-13,3916	14,17203	-0,944932	0,369369	-32,9919	6,208729
(1)X1(L)	0,2378	0,27472	0,865691	0,409143	-0,1421	0,617768
(2)X2(L)	0,3294	0,18117	1,818358	0,102370	0,0789	0,579988
(3)X3(L)	0,3591	1,42140	0,252613	0,806242	-1,6068	2,324901
(4)X4(L)	1,6794	1,10027	1,526349	0,161267	0,1577	3,201090
(5)X5(L)	0,3024	0,12114	2,495879	0,034092	0,1348	0,469901
(6)X6(L)	-0,6573	0,69715	-0,942909	0,370348	-1,6215	0,306830

Fig. 1: Results of the calculation of regression coefficients

As a result of the calculation, the following regression equation was obtained

$Y = -13.3916+0.2378X_1+0.3294X_2+0.3590X_3+1.6793X_4+0.3023X_5-0.6573X_6$

Coefficients of interaction between factors (excess effects) were not taken into account, since they are linear combinations of other effects and can not be estimated. The significance of the regression coefficients was checked by the Student t-test .The variance of the reproducibility of the regression coefficients equal to 711.75, the variance error – 180.17. For this case, we used the Student's distribution and criterion (*t*). The distribution (*t*) determines the probability that the value (*f*) is less than adequate for some selected values (*t*). The obtained data on the mathematical evaluation of the model of beeswax extraction from propolis are given in Table. 2.

Table 2: Evaluation of the model of beeswax extraction from propolis

No of variables	Criteria value			
	*t** (calculated)	*t* (C)	*P*- one-tailed (C)	*P*- two-tailed (C)
1	0.86569	0.703	0.75	0.50
2	1.81836	1.383	0.90	0.80
3	0.25261	0.129	0.55	0.10
4	1.52635	1.383	0.90	0.80
5	2.49588	2.262	0.975	0.95
6	–0.94291	0.883	0.80	0.60

The interval one-tailed estimation was determined by the formula (17) and was found from tables, depending on the *f* value

.

$$P(t) = P\{t \le t^*\} = \int_0^{t^*} P(t)dt \qquad (17)$$

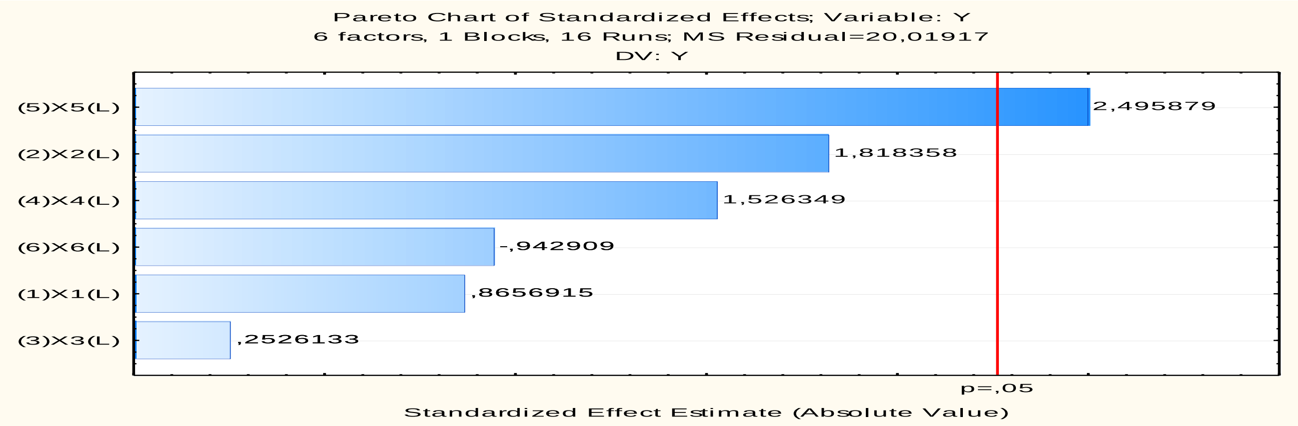
The two-tailed estimation was calculated from equation:

$$P = 2P - 1 \qquad (18)$$

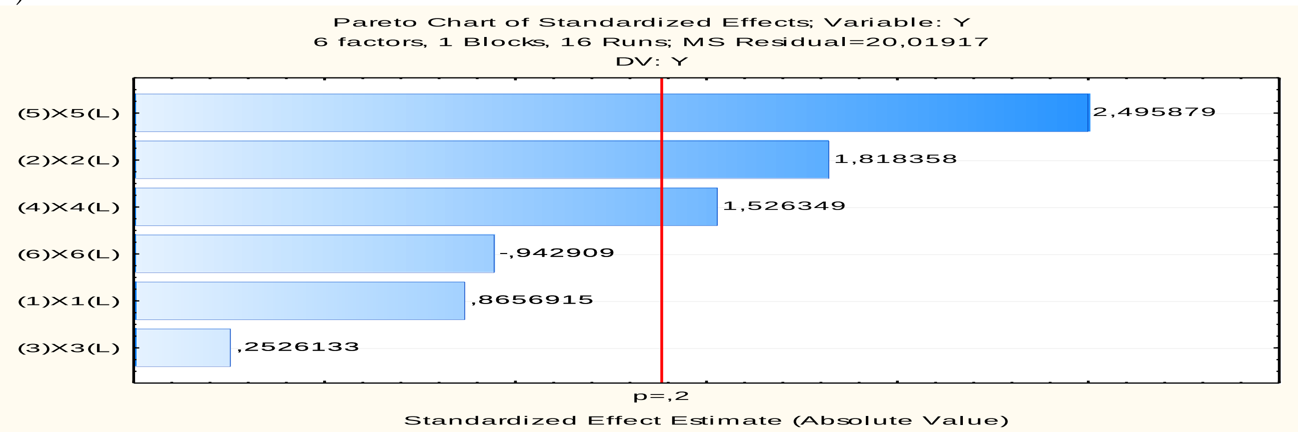
The number of the degree of freedom was found from equation:

$$f = n - m - 1 = 16 - 6 - 1 = 9 \qquad (19)$$

For the confidence figure P=0.95, only one variable is significant - the temperature (X_5) (Table 2. Fig. 2, a).If we take the confidence figure P=0.8, then a three variables X_1, X_4 and X_5 (time of single extraction, degree of grinding and temperature) are significant. If the criteria (technological parameters) are placed in a decreasing sequence, then they will have the following significance (fig. 2, b): X_5 (temperature) > X_2 (time of single extraction) > X_4 (degree of raw material grinding) > X_6 (number of stirring while the single extraction) > X_1 (quantitative ratio of propolis and solvent) > X_3 (multiplicity of extractions).



a)



B) **Fig. 2: Pareto chart of independent factors $X_1 - X_6$ for confidence figure P= 0.95 (a) and *P*= 0.8 (б).**

Model Evaluation

- In general, the multiple correlation R = 0.86421 is strong enough;
- In the table of residues, the extent of the model's correspondence in the given experiment is clearly visible (Fig. 3);
- The significance of the coefficients is shown for the variables $X_1 - X_6$.

Observed, Predicted, and Residual Values (Extraction of Vax.sta)

Observed, Predicted, and Residual Values (Extraction of Vax.sta)
6 factors, 1 Blocks, 16 Runs; MS Residual=20,01917
DV: Y

Case or Run	Observed	Predictd	Resids
1	30,00000	34,27646	-4,27646
2	20,00000	22,78406	-2,78406
3	25,00000	19,32165	5,67835
4	35,00000	35,33414	-0,33414
5	15,00000	14,45629	0,54371
6	30,00000	29,57722	0,42278
7	25,00000	23,72909	1,27091
8	27,00000	24,34281	2,65719
9	30,00000	22,91200	7,08800
10	20,00000	22,09023	-2,09023
11	23,00000	21,88529	1,11471
12	10,00000	14,70248	-4,70248
13	32,00000	28,99284	3,00716
14	25,00000	26,44301	-1,44301
15	20,00000	21,55772	-1,55772
16	15,00000	19,59470	-4,59470

Fig. 3: Observed, predicted and residual values

The adequacy of the adopted model (linear) was evaluated using the Fisher's ratio test (*F*), whose calculated value is 4.4256, and the literary value (F^* = 5.801), which corresponds to the probability $P(F^*) = 0.99$. Thus, the *F*-computed < F^*, therefore, the regression equation is adequate.

Establishment of the Optimal Technological Parameters of Beeswax Extraction from Propolis

As can be seen from the above, optimization of the technological process for the isolation of wax products from propolis represents a purposeful search for the values of the influencing factors at which the extremum of the criterion of optimality is reached (taking into account the constraints imposed on all the influencing factors and response functions).

The solution of tasks in hand depends on the area of variation of the independent and dependent variables; the starting point of the search; type of the regression equation. The using of the simple method (SM) for the solving of the resulted regression equation (MS Excel 2016, Math Cad 14), given the constraints of factors (table 3), did not yield satisfactory results. Based on the results of the experiment, it can be assumed that to further optimization of the technological parameters the application of the "steepest ascent" method will be effective, since the resulted linear model is adequate and not sharply asymmetric comparative to coefficients. Calculation of the "steepest ascent" was carried out in such sequence. The value of the step of motion along of the gradient concentrations of factors in the medium was calculated according to standard procedure, starting from the values of the regression coefficients.

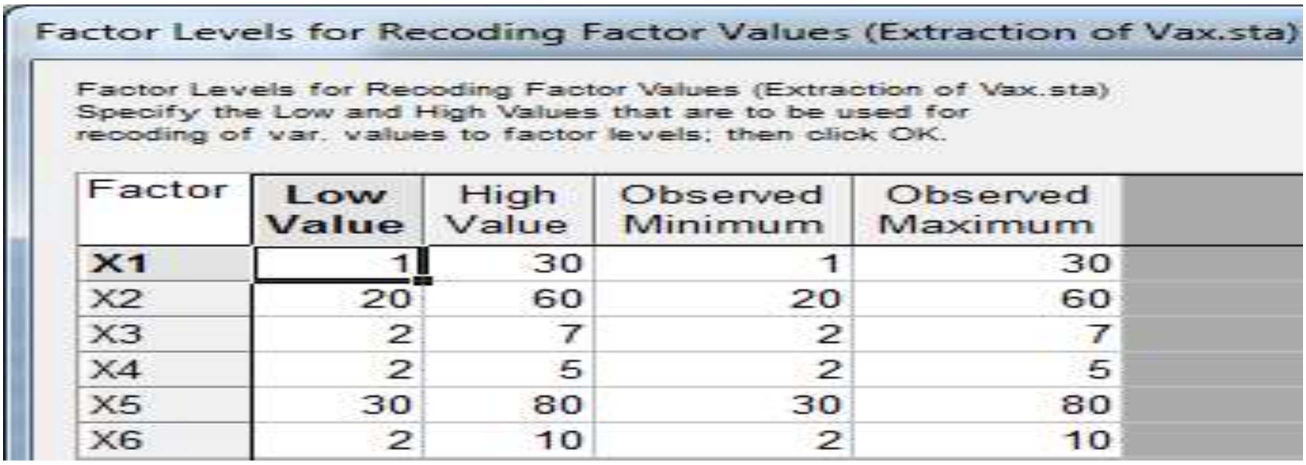
The factor with the maximum effect (X_5) was taken as the base one, and the product of the corresponding regression coefficient was calculated for the variation step ($b_i \Delta X_i$), in this case – $b_5 \Delta X_5$. Then, the motion step ΔX_i^* for the basic factor was selected, with which the optimization of the technological process will be carried out (usually $\Delta X_i^* \leq \Delta X_i$), then the ratio was calculated:

$$\gamma = \frac{\Delta X_i^*}{b_i \Delta X_i} \quad (20)$$

For all other factors, the motion steps to the optimal values were calculated using the formula:

$$\Delta X_j^* = \gamma - b_j \Delta x_j \quad (21)$$

The movement to the optimum values began from the center of the plan, which was used to obtain a mathematical description of the response function. Values of factors at each new step were found by adding ΔX_j^* to the corresponding previous values. The movement to the optimum is stopped usually in two cases, namely: if the value of one, several factors or response functions have reached to limits of admissible values and if the extremum of the criterion of optimality (Y) is reached, which was done by us in the conducted experiment. In the planned experiments it was required to achieve the optimum of beeswax extraction under the following restrictions to the main technological parameters determined experimentally: $1 \leq X_1 \leq 30$; $1 \leq X_2 \leq 60$ min; $1 \leq X_3 \leq 10$ times; $1 \leq X_4 \leq 6$ mm; $20 \leq X_5 \leq 80$ ° C; $0 \leq X_1 \leq 10$ times (Fig. 4).


Factor Levels for Recoding Factor Values (Extraction of Vax.sta)

Factor Levels for Recoding Factor Values (Extraction of Vax.sta)
Specify the Low and High Values that are to be used for recoding of var. values to factor levels; then click OK.

Factor	Low Value	High Value	Observed Minimum	Observed Maximum
X1	1	30	1	30
X2	20	60	20	60
X3	2	7	2	7
X4	2	5	2	5
X5	30	80	30	80
X6	2	10	2	10

Fig. 4: Limitations of the dependent factors based on the results of STATISTICA package calculations

Calculations according to this algorithm were carried out using the Excel spreadsheet package MS Office 2016 Professional Plus (Ver.1806) [25] (Table 3, Fig. 5).

Table 3: Initial data to calculations for optimization of conditions while beeswax products extracting from propolis

Factors	Limitation of factors	Correlation coefficient	Middle value	Regression coefficient, b_i
X_1	1 ÷30	–0,39495	11,125	0,238
X_2	1 ÷60	0,58445	42,187	0,329
X_3	1 ÷ 10	0,17442	4,687	0,359
X_4	1 ÷ 6,0	–0,06705	3,500	1,679
X_5	20 ÷80	0,76635	55,625	0,302
X_6	0 ÷10	–0,33497	5,563	–0,657

$b_0 = -13.392$

	X_1	X_2	X_3	X_4	X_5	X_6	Y
b_i	0,238	0,329	0,359	1,679	0,302	-0,657	
ΔX_i	1	1	1	1	10	1	
$b_i * \Delta X$	0,238	0,329	0,359	1,679	3,02	-0,657	
γ	0,788	1,089	1,189	5,560	0,302	-2,175	
$\Delta Xi*$	3,073	2,982	2,952	1,632	3,311	-2,654	
Base	11,125	42,187	4,687	3,500	55,625	5,563	23,838
Step -1	14	45	8	5	59	3	32,10
Step -2	17	48	11	7	62	0	40,35
Step -3	20	51	14	8	66	-2	48,61

Response surface methodology

Fig. 5: Results of calculations of "steepest ascent" in MS Excel

According to experimental results, represented in Table.5, it was found that already at the 2nd step, the value of X_6 reached a minimum at the maximum value of Y. In this connection, the further continuation of calculation is inexpedient.

Thus, using the methods of mathematical planning, the optimal conditions for the technology of purified wax obtaining from propolis have been determined.

The maximum extraction can be carried out under the following conditions: X_1 (quantitative ratio of propolis and solvent) is 1:17; X_2 (time of single extraction) is 48 min; X_3 (extraction multiplicity) is 11 times; X_4 (degree of raw materials grinding) is 7 mm; X_5 (temperature) is 62 °C; X_6 (number of stirring) is 0. On the basis of the above, the calculation conducted by the "steepest ascent" method is more effective than the calculations obtained by the simplex-method.

Conclusions

- According to modern literary sources, it is established that propolis is an economically accessible natural source of raw materials for purified beeswax obtaining.
- The technology of purified beeswax obtaining from propolis was developed by the method of mathematical design of experiment; it meets a modern physical-chemical and therapeutic requirements and has the yield of beeswax about 40 %.
- The optimal conditions for the technology of purified beeswax obtaining from propolis were determined by the "steepest ascent" method. The maximum extraction can be carried out under the following conditions: X_1 (quantitative ratio of propolis and solvent) is 1:17; X_2 (time of single extraction) is 48 min; X_3 (extraction multiplicity) is 11 times; X_4 (degree of raw materials grinding) is 7 mm; X_5 (temperature) is 62 °C; X_6 (number of stirring) is 0.

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