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The Influence Of Technological Parameters On The Process Of Co2-Extraction Of Biologically Active Substances From Licorice Root

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ABSTRACT

The aim of the work is to experimentally study the effect of pressure, temperature and time of supercritical CO_2 (SC-CO_2) extraction on the yield of extractives from local raw materials - licorice root.

The object of the study was the roots of naked licorice grown in the vicinity of the Bukhara region (border with Turkmenistan).

The experiments were carried out in apparatus for SC-CO_2-extraction in the laboratories of the Bukhara Engineering-Technological Institute and the Kuban State Technological University.

The main factors influencing the extraction process of licorice root are taken as follows: T -is the temperature of the extractant (0C), τ is the duration of extraction (min), P is the pressure of the extractant (MPa).

The experimental conditions were as follows: pressure 7,5-9,5 MPa, temperature from 310C to 410C, extraction duration 90-150 minutes. The extract yield Yyeld (%) is taken as the sought function.

The implementation of the intensified process of licorice root extraction is associated with obtaining calculated equations that allow determining the duration of the extraction process and rational parameters of the extraction mode. For this purpose, we have applied the method of planning multifactor experiments.

A generalized equation has been determined that describes the process of CO2 extraction of licorice roots, with the help of which it is possible to reveal the degree of influence of each investigated factor (extractant temperature, process duration, extractant pressure) on the final result, which is necessary to optimize the process.

KEYWORDS

Planning multifactor experiments, biologically active substances, licorice root, extraction, extract, supercritical carbon dioxide, pressure, temperature, extraction time.

INTRODUCTION

Licorice (Glycyrrhizaglabra L.) is a perennial shrub of the legume family that grows mainly in Central Asia, the Middle East and Southeastern Europe. Since ancient times, dried licorice roots have been widely used as a food flavoring agent and for various medicinal purposes [1,2,3,4,5,6].

There are about 13 types of licorice root in the world. The most common types are: naked licorice (Glycyrrhizaglabra), Ural licorice (Glycyrrhizauralensis Fisch) and Korzhinsky licorice (Glycyrrhizae Korshinskyi Grig). Naked licorice is the most popular among them, its roots contain the largest amount of biologically active substances (BAS) [7,8,9,10]. The quality of licorice root is standardized by the State Pharmacopoeia: the content of extractive substances (ES) extracted by 0,25% ammonium hydroxide solution must be at least 25%, moisture no more than 14%, ash no more than 8%, glycyrrhizic acid (GA) - no less 6% [11,12,13,14]. GA is the most valuable extractable substance; however, licorice root contains other biologically active substances: up to 5,0 % of flavonoids, carbohydrates up to 34,0%, proteins up to 10,1%, amino acids up to 12,71%, including asparagine up to 4,0%, fats and fat-like substances up to 4,7%, ascorbic acid up to 3,1% [15,16,17].

Known technology of extraction of licorice root [18], in which the extraction consists in the process of bimaceration with 0,25% aqueous ammonia solution with a modulus of 1: 5 for 48 hours, the second time - with a threefold amount for 24 hours.

In the conditions of specialized liquorice factories, the hood is obtained using a battery of diffusers operating on the counterflow principle. Extraction is carried out with hot water [19]. The technological sequence for the production of licorice root extract is as follows: grinding; extraction in batch diffusers; filtration; evaporation of juices in boilers; thickening of the extract in the evaporator of the I-group; thickening of the extract in an evaporator of the II-group; packaging, labeling, storage.

The disadvantage of these technologies is the low yield of the extract, the long duration and energy consumption of the process.

MATERIALS AND METHODS

There is a vacuum-pulse technology for processing licorice root. The principle of operation of this extraction [20] is based on preliminary degassing of raw materials under vacuum, impregnation with an extractant under atmospheric pressure, periodic heating, followed by impulse evacuation to a residual pressure equal to the vapor pressure of the solvent at a given temperature, and connection with atmospheric pressure. As a result of impulse evacuation, the solvent boils up in the pores of the material, and the resulting vapor pushes the extractant saturated with the target component into the volume of the miscella. Then, when combined with the atmosphere, fresh portions of the solvent enter the pores of the material. The above sequence of actions is repeated as many times as necessary.

The use of ammonia in this technology creates some technical problems, since it, being an aggressive medium, will volatilize during evacuation and adversely affect pumps, the inner surface of pipelines, receivers. In addition, its inevitable emissions into the environment are unacceptable.

In work [21], the ammonia solution in vacuum-pulse extraction was replaced by sodium hydroxide, which is not subject to evaporation. The extraction time of licorice root at a temperature of 60°C, an alkali concentration of 0,5% of the mass. and in a pulse mode with a cycle of 10 minutes does not exceed two hours. Prospective for extraction is the use of carbon dioxide in subcritical regions (at pressures below 7,39 MPa) (liquefied state) and supercritical regions (compressed state), the so-called CO_2-extraction. Carbon dioxide goes into a supercritical state at critical temperature (31,30C) and pressure (7,39 MPa). In this state, the substance expands, occupying the entire provided volume, like a gas, but has a high density, like a liquid. Thus, supercritical CO_2 can, in principle, better than a classical solvent penetrate into the material to be extracted, absorb and transport dissolved components [22, 23, 24].

In [25], supercritical extraction with carbon dioxide (SC-CO_2) of glycyrrhizin from licorice root was performed. The composition of the solvent and the particle size were investigated in order to obtain the maximum yield. The optimal extraction conditions were as follows: pressure 30 MPa; temperature 50°C; CO_2 consumption about 10 kg / h; particle size 0,3-0,4 mm. The SC-CO_2 extraction was better than traditional extraction, ultrasonic extraction, microwave extraction and so on.

Traditionally, licorice root is most actively grown (due to climatic features) in the north-west of Uzbekistan - in Karakalpakstan and the Khorezm region. According to foreign experts, licorice root is mainly exported in the form of raw materials [26,27]. Therefore, the technological processing of this local valuable medicinal raw material and the production of a CO_2 extract is urgent. CO_2-extract is a concentrate of the plant's own substances, free of impurities, solvents and water, obtained using carbon dioxide as a solvent.

The aim of this work is to experimentally study the influence of pressure, temperature, and time of CO_2-extraction on the yield of extractives from local raw materials - licorice root.

Materials and methods. The object of the study was the roots of naked licorice grown in the vicinity of the Bukhara region (border with

Turkmenistan). The quality of the roots meets the requirements of GOST 22839-88 Roots and rhizomes of licorice. Technical conditions. Licorice roots and rhizomes. Specifications. The yield of the obtained extract was determined according to the requirements of GOST 22840-77 (date of update of the description: 06.01.2019) Licorice root extract. Technical conditions. Extract of licorice root. Specifications.

The licorice roots were cut into strips and ground in an electric mill to a size of 0,6-0,8 mm. The experiments were carried out on laboratory facilities, the description of which is given in [28,29].

The implementation of the intensified process of licorice root extraction is associated with obtaining calculated equations that allow determining the duration of the extraction process and rational parameters of the extraction mode. For this purpose, we applied the method of planning multifactor experiments [34,35,36,37].

Experimental planning methods allow the most economical and efficient way to obtain mathematical models of the investigated process in the realized range of changes in many factors influencing the process.

It is possible to get a clear idea about the place of planning experiments if we consider the general scheme of paired experimental research of an object with an insufficiently clarified mechanism of the processes occurring in this object.

EXPERIMENTAL PART

The main factors influencing the extraction process of licorice root are taken as follows: T - is the temperature of the extractant (0C), τ - is the duration of extraction (min), P - is the pressure of the extractant (MPa).

The limits of variation of the factors are selected based on the analysis of the results of preliminary experiments to study the process of CO_2 extraction of plant materials.

The extract yield Yyeld (%) is taken as the sought function.

In our case, when the number of factors is three, when implementing the experimental plan, according to [34], it is necessary to carry out 15 experiments, varying the factors at five levels.

Allocation of factors and levels of their variation is presented in table. 1.

Tab. 1. Levels of the studied factors

№	Factors	Levels				
		1	2	3	4	5
1	Extractant temperature, T (C^o)	31	33	35	38	41
2	Extraction time, τ (мин)	90	105	120	135	150
3	Extractant pressure, P (МПа)	7,5	8,0	8,5	9,0	9,5

RESULT AND DISCUSSION

Experimental results. The implementation of the 5×3 experiment plan with the values of the factors is presented in table. 2.

Tab. 2. Results of the experiment plan reproduction

№	T (C°)	τ (min)	P (MPa)	Y_{yeld}, %
1	31	90	7,5	26,6
2	31	105	8	28,7
3	31	120	8,5	32,4
4	31	135	9	33,2
5	31	150	9,5	34,4
1	35	90	8,5	28,1
2	35	105	9	33,7
3	35	120	9,5	31,6
4	35	135	7,5	30,4
5	35	150	8	34,7
1	33	90	9,5	33,9
2	33	105	7,5	26,7
3	33	120	9	34,1
4	33	135	8,5	33,2
5	33	150	8	31,4
1	41	90	8	28,4
2	41	105	8,5	29,9
3	41	120	9	31,2
4	41	135	9,5	34,4
5	41	150	7,5	33,8

№	T (C°)	τ (min)	P (MPa)	Y_{yeld}, %
1	38	90	9	31,6
2	38	105	9,5	32,8
3	38	120	7,5	28,7
4	38	135	8	31,4
5	38	150	8,5	33,5

The structure of the matrix is such that during all experiments, each level of any factor meets once with each level of all other factors, for this each level of each factor is set in the experiments as many times as the levels are accepted. This achieves the averaged effect of any factor, i.e. The same effect is provided that occurs with an infinitely large number of experiments with a random variation of all factors. This opens up the possibility of applying the methods of mathematical statistics and achieves savings in the number of experiments [34].

From table. 3 it can be seen that the average values of the five levels of each function are equal to the general average: Yav.yeld = 32,5%. The coincidence of the mean with the total mean is a criterion for the absence of errors in calculations.

Empirical formulas for the formulation of partial dependences of the extract yield, final temperature and final pressure depending on the extraction parameters, initial moisture content of plant material, temperature and CO_2 flow rate were obtained as a result of data processing by the least squares method [34,38].

Tab. 3. Experimental values of partial functions

Functions	**Levels**					**Average value**
	1	**2**	**3**	**4**	**5**	
Y_1(T)	31,92	33,04	33,32	33,18	32,02	32,5
Y_2(τ)	30,6	32,64	32,7	33,38	33,16	32,5
Y_3(P)	31,7	31,84	32,14	32,78	34,02	32,5

Formulas and calculated values of partial functions are presented in Table 4.

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Tab. 4. Calculated values of partial functions

Functions	Levels					Average value
	1	2	3	4	5	
$y = 41{,}772 + 4{,}176 \cdot x - 0{,}058 \cdot x^2$	31,98	32,9	33,38	33,2	32,0	32,6
$y = 10{,}537 + 0{,}336 \cdot x - 0{,}0001 \cdot x^2$	30,77	32,19	33,05	33,36	33,11	32,5
$y = 75{,}080 - 0{,}2211 \cdot x + 7257 \cdot x^2$	31,74	31,76	32,13	32,87	33,97	32,5

Graphs of dependences of the extract yield on influencing factors are shown in Fig. 1-3.

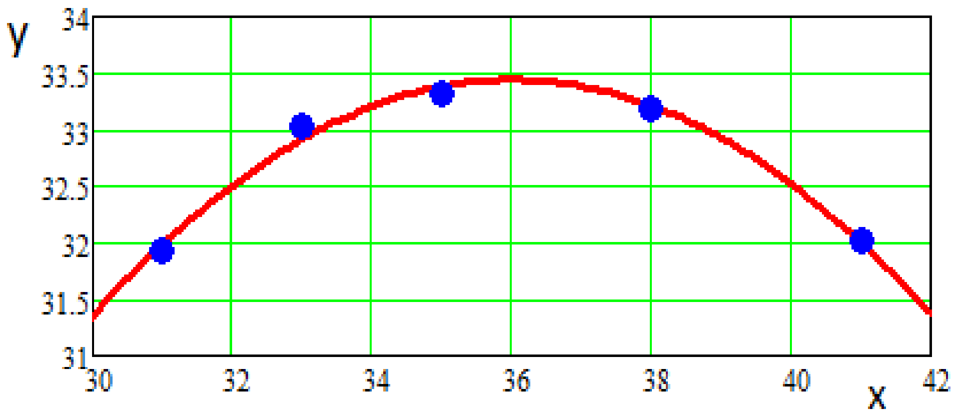

Fig. 1. Dependence of the extract yield on the effect of temperature according to the model

$$y = -0.058 \cdot x^2 + 4.176 \cdot x + 41.772$$

Points - are experimental data

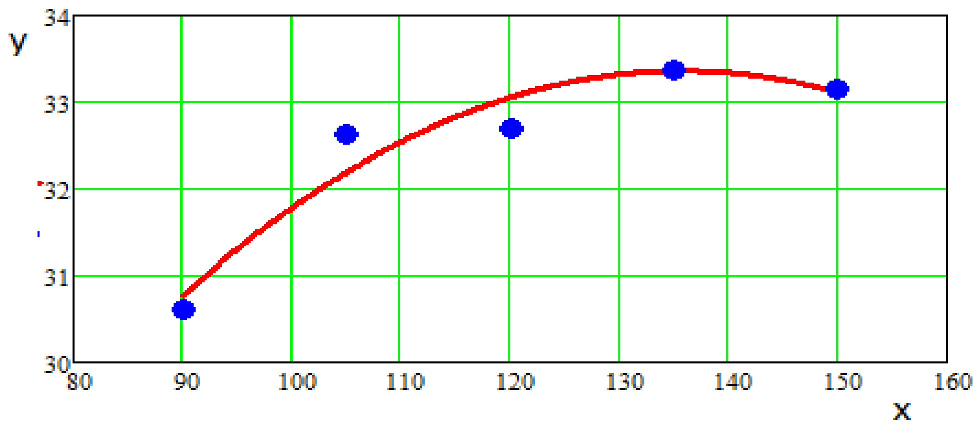

Fig. 2. Dependence of the extract yield on the influence of the extraction time according to the model

$$y = -0.0001 \cdot x^2 + 0.336 \cdot x + 10.537$$

Points – are experimental data

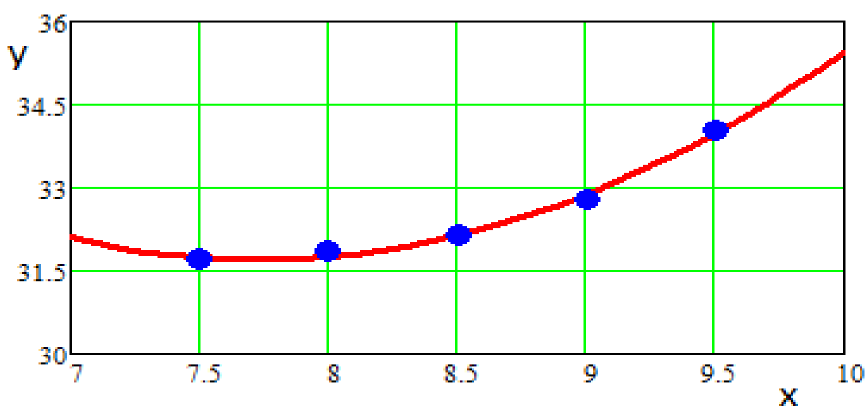

Fig. 3. Dependence of the extract yield on the influence of model pressure

$$y = 0.7257 \cdot x^2 - 0.2211 \cdot x + 75.08$$

Points - are experimental data

Determination of the significance of private functions. In order to make a judgment about the degree of validity of the adopted parameters, we determined their significance. In accordance with

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the provisions of mathematical statistics and probability theory, functions describing the process are divided into significant and insignificant. If the function is insignificant, then the interval of its change does not go beyond the admissible scatter of experimental data, i.e. is within the confidence interval.

We determine the significance of partial dependence functions using the coefficient of nonlinear multiplier correlation R [34]:

$$R = \sqrt{1 - \frac{(N-1)\cdot\sum_1^N (Y_{exp} - Y_{cal})^2}{(N-k-1)\cdot\sum_1^N (Y_{exp} - Y_{av})^2}} \qquad (1)$$

where N - is the number of described points (N = 5); k - is the number of operating factors (k = 1); Y_{exp} - experimental result; Y_{cal} - theoretical (calculated) result; Y_{av} - average experimental value.

Tab. 5. Values calculated from calculated data

Functions	**Levels**					**Amount**
	1	2	3	4	5	
$(T)=(Y_{exp}- Y_{cal})^2$	0,0322	0,1594	0,025	0,0084	0,0658	0,2908
$(P)= (Y_{exp}- Y_{cal})^2$	0,0278	0,2038	0,1247	0,0003	0,0024	0,0718
$(\tau)= (Y_{exp}- Y_{cal})^2$	0,0019	0,0069	0,0005	0,0086	0,0020	0,019452

Table 6. Values calculated from the average calculated data

Functions	**Levels**					**Amount**
	1	2	3	4	5	
$(T)=(Y_{exp} – Y_{av})^2$	0,6	0,1183	0,3893	0,2342	0,4569	1,8
$(P)= (Y_э- Y_{av})^2$	2,9899	0,0945	0,3104	0,7475	0,378	4,52
$(\tau)= (Y_э- Y_{av})^2$	0,9920	0,7327	0,3091	0,0071	1,7530	3,7939

The significance is determined by the formula [34]:

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$$t_R = \frac{R\sqrt{N-k-1}}{1-R^2} \quad (2)$$

The values of the correlation coefficients R and its significance t_R are given in Table 7.

Tab. 7. Correlation coefficient R and its significance t_R for partial functions

Function	R	t_R	Function value
$Y_1(T)$	0,988	73,5 › 2	significant
$Y_2(P)$	0,95	15,46 › 2	significant
$Y_3(\tau)$	0,99	237› 2	significant

To obtain the equation of the technological process of CO_2 - extraction of licorice roots, the formula proposed by M.M. Protodyakonov [34]:

$$Y_P = \frac{\prod_{i=1}^{n} Y_i}{Y_{av}^{n-1}} \quad (3)$$

where Yp - is a generalized function; Y_i - is a partial function; $\prod_{i=1}^{n} Y_i$ - product of partial functions; Y_{av}^{n-1}- the total average of all the values of the generalized function taken into account, in power by one less than the number of partial functions.

According to formula (3), generalized equations describing the process of supercritical CO_2 extraction of licorice roots are defined:

$$Y_{yeld} = \frac{(41{,}772+4{,}176\cdot x-0{,}058\cdot x^2)*(10{,}537+0{,}336\cdot x-0{,}0001\cdot x^2)}{32{,}49^2*(75{,}080-0{,}2211\cdot x+7257\cdot x^2)^{-1}} \quad (4)$$

The inference results and ratios (4) are presented in table 8.

Tab. 8 Inference Results and relations (4)

Y_{yeld}, %	Y_{cal}	$\lvert Y_{exp}- Y_{cal}\rvert$	$\lvert Y_{exp} – Y_{av}\rvert$	$\lvert Y_{exp} - Y_{cal}\rvert^2$	$\lvert Y_{exp} - Y_{av}\rvert^2$
26,6	31,38	3,70	5,89	13,70	34,69
31,7	32,49	0,42	0,79	0,17	0,62
31,4	31,93	0,79	1,09	0,63	1,18
35,5	33,61	1,02	3,01	1,05	9,06
34,4	33,05	0,01	1,91	0,0002	3,64
31,6	32,49	0,37	0,89	0,13	0,79
32,7	31,93	1,28	0,21	1,64	0,04
35,2	33,61	1,47	2,71	2,17	7,34
32,4	33,05	1,33	0,09	1,78	0,008
34,7	31,38	2,18	2,21	4,77	4,88
31,9	31,93	1,13	0,59	1,28	0,34
34,6	33,61	1,45	2,11	2,12	4,45
34,1	33,05	0,85	1,61	0,73	2,59
33,2	31,38	1,09	0,71	1,20	0,50
31,4	32,49	2,35	1,09	5,52	1,18
31,3	33,61	0,79	1,19	0,63	1,41
31,4	33,05	0,91	1,09	0,83	1,18
31,2	31,38	0,08	1,29	0,007	1,66
34,4	32,496	1,43	1,91	2,07	3,64
31,8	31,938	1,08	0,69	1,18	0,47
31,6	33,054	0,06	0,89	0,004	0,79
32,8	31,38	2,03	0,31	4,12	0,09
31,6	32,496	0,89	0,89	0,81	0,79

Y_{yeld}, %	Y_{cal}	$\|Y_{exp}- Y_{cal}\|$	$\| Y_{exp} – Y_{av}\|$	$\| Y_{exp} - Y_{cal}\|^2$	$\| Y_{exp} - Y_{av}\|^2$
31,4	31,938	1,09	1,09	1,19	1,18
33,5	33,612	1,21	1,01	1,47	1,02

Based on the tabular data, the correlation coefficients for the generalized equation (4) are found.

For equation (4), the correlation coefficient for temperature T is R = 0,67 and its significance tR = 2,105, for pressure R = 0,77 and its significance tR = 3,386, for the extraction time R = 0,87 and its significance tR = 2,012. The data presented indicate the adequacy of the generalized equation (4).

CONCLUSIONS

The resulting equation (4) makes it possible to determine with sufficient accuracy the yield of extractives from licorice roots of local origin under the conditions of SC-CO2-extraction in the investigated range of changes in factors. Using equation (4), it is possible to identify the degree of influence of each investigated factor (extractant temperature, process duration, extractant pressure) on the final result, which is necessary to optimize the process.

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