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THE MODELING OF OPTIMIZATION OF SUPERCRITICAL CO_2 EXTRACTION OF RESVERATROL FROM BERRIES OF MULBERRY

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ABSTRACT

This study used a supercritical (SK) of CO_2 - extraction to extract resveratrol (RT) from the mulberry. To do this, define the conditions (preliminary experiments) of the extraction process, namely, the temperature, pressure and fluid flow SC (CO_2). Given that this process is multifactorial, the method RSM - response surface methodology and CCRD -central composite rotatable design used to determine the optimum operating conditions of the process. The effectiveness of the established SC-CO_2 extraction conditions, expressed RT content in the extracts as compared with a yield of RT produced by the conventional extraction method, when applied SC-CO_2 modified polar co-solvent (ethanol). In describing the RT yield predictions using appropriately combined RSM with CCRD, we found that the yield of RT mainly depends on the pressure and quantity of SC-CO_2 used for extraction. It turned out that there is a significant relationship for the linear and quadratic terms of the relationship between the output of the RT and these parameters. Noticeable interaction between the three process parameters (pressure, SC-CO_2 temperature and flow rate) was observed. Mulberry is subjected to heat pre-treatment. Cooked thereafter pitch used as a raw material for the extraction of by SC-CO_2. Initial studies for a wide spectrum of SC-CO_2 density value (690-780 kg / m^3) indicates that it is possible to set optimum operating conditions for the RT isolation. According to RSM - analysis of the optimal process conditions: 15,8 MPa, 30,5^0 and 20,08 g CO_2 / g.d.m CO_2 consumption for the extraction of RT from licorice using SC-CO_2. SC-CO_2 density calculated for the optimum pressure and temperature equal to 725 kg / m^3, which was found as a result of a preliminary analysis of the correlation between the output of the RT and CO_2 density. The maximum yield of RT is equal to 0,052 g of 1 g of dried material (about 0,5% of extract) with SC-CO_2 density equal 725 kg/m^3.

Preliminary tests performed at condition resulting in SC-CO_2 density ranging from 690 to 780 kg/m^3 indicated that at some pressure, temperature as well as consumption of supercritical fluids the optimal working conditions for resveratrol isolation could be determined. For this purpose the following range of working conditions of SK-CO_2 were tested by using Central Composite Rotatable Design (CCRD) and Response Surface Methodology (RSM): pressure from 18 to 30 MPa, temperature from 20^0 to 40^0 and consumption of SK-CO_2 from 12 to 24 $g_{CO_2}/g_{d.m}$. The results of this investigation indicated that maximum yield RT 0,052 mg from 1 g materials on dry basis (about 0,5 % of total extract) at 15,8 MPa, 30,5^0 and 20,08 $g_{CO_2}/g_{d.m}$ could be obtained.

KEYWORDS: *Berries of mulberry, organic acids, glucose, fructose, essential oils, vitamins, carotenes, micro- and macro elements, resveratrol.*

INTRODUCTION

Mulberry berries (white, red and black) contain a large number of substances useful for the human body: simple sugars (glucose and fructose), organic acids, essential oils, vitamins (water- and fat-soluble), carotenes, a number of micro- and macro elements. The content in the fruits of the strongest natural antioxidants - carotenes, vitamins C, E, resveratrol and selenium - relieves the aging organism of many diseases and has a rejuvenating property.

The purpose of this study was to analyze the chemical composition of fruits and leaves of mulberry, as well as the isolation of individual ingredients, in particular resveratrol (PT), using extraction with supercritical carbon dioxide (CO_2).

In previous works, conditions of supercritical (SC) -$C0_2$ extraction of glycyrrhizic acid (GA) from licorice roots were considered. To do this, it was necessary to determine, by preliminary experiments, the conditions of the extraction process, namely, the temperature, pressure, and flow rate of SC-CO_2. Bearing in mind that this process is multifactorial, RSM - response surface methodology and CCRD - central composite rotatable design were used in the work with the aim of establishing optimal operating conditions of the process [1,2]. A similar approach was used by other authors [3] in the extraction of diosgenin (DG).

Advantages of the SC-CO_2 extraction method, as compared to conventional methods, are already known [4]. SC - extract does not contain a solvent, the extraction process can be carried out very quickly and finally, there is no need for further purification of the SC extract from the extractant.

EXPERIMENTAL PART

Extraction was carried out in weighing bottles autoclave firm ASTELL (UK), the extracts were collected in receptacles to determine their total content. Samples were stored in the refrigerator (-5^{0}C) for analysis RT by liquid chromatography high resolution (HPTLC, ELSA company Ing–Techn, 2000 UK).

The effect of the duration of SC-CO_2 extraction was studied at 32°C and 16 MPa. Samples of the extracts were collected after different extraction times, which corresponded to a specific flow rate of the SC fluid. The yield of the extract was compared and used for subsequent analysis of the effect of operating conditions [5,6].

To study the effect of pressure, temperature and extraction duration (SC-CO_2 flow), as well as their interactions, RSM and CCRD were used. A similar procedure was applied earlier [1]. However, instead of comparing the output of diosgenin (DG) SC-CO_2 extraction was determined using the "overlapping" parameters (yield DG received SC-CO_2 was compared with the yield by the method [2]) as a dependent variable [1], total extract yield on gram of the prepared isolate and the content of DG in 100 g of a common isolate or per 100 g of a dry plant were used in this work. The investigated parameters were pressure (denoted as x_1, MPa, the temperature (x_2, 0 ° C) and the amount of consumed CO_2 (x_3, mCO_2 / mGA). Actual and coded variables used in the experimental scheme, determined on the basis of preliminary experiments carried out for various densities –SC-CO_2. The central building experience are 3 variables and all 20 experiments involving nine factorial vertices 5 and 6, the center points for RSM and CCRD analysis used yield as shown in Table. 1. Polinomal equation 2nd order prediction considered yield RT as a function of the independent (i = 3) pressure (x_1), temperature (x_2) and the amount of CO_2 (x_3) as an encoded value

$$\gamma = \sum \beta_0 + \sum \beta_i x_i + \sum \beta_{ij} x_i^2 + \sum \sum \beta_{ij} x_i x_j \quad (1)$$

It true for j<1 and i = 3.

The Matlab 2014 version was used to apply RSM analysis of experimental data with a 3-dimensional surface dependence and a contourmap of independent variables and their interactions [7].

Table. 1. Data on the composition of mulberry fruits according to USDA Nutrient Database are presented.

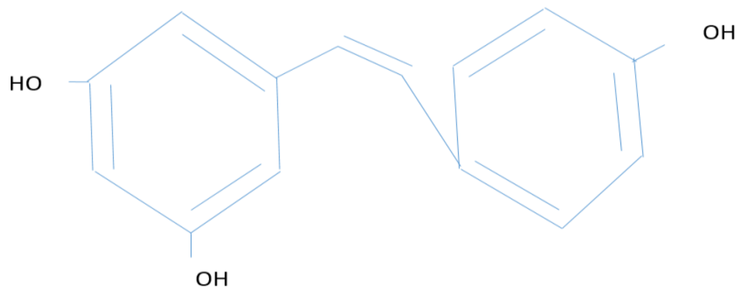
TABLE 1 COMPOSITION OF MULBERRY FRUITS PER 100 G OF FRUIT

1	Organic acids	1,2 g.
2	Mono- and disaccharides	8-12 g.
3	Vitamin B_1 (thiamine)	0,04 mg.
4	Vitamin B_2 (riboflavin)	0,01 mg.
5	Vitamin PP (niacin)	0,80 mg.
6	Vitamin C (ascorbic acid)	30 mg.
7	Vitamin B_4 (choline)	12 mg.
8	Vitamin A (retinol)	6 mkg.
9	Carotene (vitamin A provitamin)	0,02 mkg.
10	K_1 (phylloquinone)	8 mkg.
11	Lutein	130 mkg.
12	Antioxdants	20 mkg.
13	Resveratrol	40 mkg.

A comparative study of amino acid extracts of leaves of white, black and red mulberry showed that their total content in black mulberry is 13.10 g.%, White - 10.60 g.% and red - 9.4 g.%.
The first part of the research is devoted to the study of the effect of different density of SC-CO_2 on the total yield of the extract. Based on the preliminary data obtained, as well as tests at various pressures, temperatures and flow rates of SC-CO_2, we tried to establish optimal operating conditions for the isolation of RT by SC-CO_2 extraction. Studies concerning the effect of

pressure, temperature, and density of SC-CO_2 on the yield of PT, as well as the interaction of these parameters during the process have not been carried out so far. It turns out that the selectivity of SC-CO_2 extraction can be achieved using different pressures, temperatures and flow rates of SC-CO_2 (3).

Resveratrol refers to polyphenols, solubility in water 0.003 g / 100 ml.



The content of the material is 0.2-5.8 mg / l.

Scheme of Experiments

Based on preliminary studies, the following network of coded parameters (x1, x2, x3) and non-coded parameters (X_1 - pressures, MPa, X_2-temperature, ^{0}C and X_3, SC-CO_2, gCO_2 / gdm) were selected, as shown in Table 2.

TABLE 2. NOT ENCODED AND CODED VALUES OF INDEPENDENT VARIABLES USED IN THE RSM-SCHEME.

Coded values	P_1 (MPa)	t, ^{0}C	flow rate (rCO_2 / r_{sm})
-1,598	8,2	20	10,68
-1	10	24	14
0	12,5	30	18
+1	15	36	22
+1,598	16,8	40	26,6

A) the density of SC-CO_2 690-780 kg/m^3
B) t = 30^{0}C is the center point, ± 100

C) P_{kr} = 7.41 MPa

The rotatable scheme was applied for the above independent variables (pressure, temperature, CO_2 flow), which was carried out 20 times.

Influence of the density of SC-CO_2 and time.

It turned out that the total yield of the extract could be increased almost 2-fold if the extraction time of SC-CO_2 increased from 80 min (CO_2 flow rate = 12 gCO_2 / g.dm) to 150 min (CO_2 flow 22 gCO_2 / g.dm). It is interesting that if further extension of the extraction time continues and finished after 180 minutes, only an additional (10%) increase in the yield of the extract is achieved. This fact was the main use for 150 min. Duration of SRE (CO_2 flow rate 22 gCO_2 /

g.dm.), at which the yield of the total extract for various densities of 690-780 kg / m^3 was analyzed).

For the SC-CO_2 density used above 780 kg / m^3, the extract yield was greater than 12% and about 780 kg / m^3 the maximum yield (15%) was achieved. Although the relationship between the yield of the total extract and the density of SC-CO_2 indicates that some maximum yield could be at a density of about 780 kg / m^3, it is more realistic to assume that the yield could be higher for a density above 780 kg / m^3 and, that with an increase in density above 780 kg / m^3, the yield of the initial total extract should be between 12 and 15% (g_{exp} / 100 g.dm).

According to preliminary experiments, although there was a limited amount (4), it is evident that the desired operating conditions for optimum recovery could be established for the SC-CO_2 density higher than 780-840 kg / m^3 and lower than 788 kg / m^3. This conclusion follows from experiments carried out at the same duration (150 min) and further details and a more accurate analysis of pressure and temperature (including the density of CO_2) as well as the effect of the flow rate of SC-CO_2 (120 mm, the extraction time) is performed using RSM and CCRD.

RSM and CCRD Analysis

The rotatable scheme was applied 20 times for the three above variables (pressure, temperature, CO_2 flow).

The results of determining the total yield of the extract are shown in Table. 3.

TABLE 3.THE TOTAL YIELD AND SEPARATION OF RT BY SC-SO_2 EXTRACTION, CARRIED OUT UNDER VARIOUS CONDITIONS

№	Pressure, (encodingvalue) x_1	Temperature (encoding value)x_2	Amount of sample. CO_2 (kg / m^3)	Density of CO_2 (kg/m^3)	Extract output (r_{exp}/r_{sm})	Output of RT mg/*r.d.m*
1.	+1	+1	+1	780	80,4	122
2.	+1	+1	-1	780	70,8	108
3.	+1	-1	+1	832	70,8	114
4.	+1	-1	-1	832	60,6	98
5.	-1	+1	+1	660	50,6	95
6.	-1	+1	-1	660	50,2	84
7.	-1	-1	+1	750	60,6	95
8.		-1	-1	750	26,2	42
9.	+1,678	0	0	830	18,8	28
1(	-1,678	0	0	650	17,8	26
1		+1,678	0	710	94,2	146
1		-1,678	0	824	88,4	130
1		0	+1,678	782	88,4	130
1		0	-1,678	782	52,2	85

15		0	0	690	80,6	130
16		0	0	690	80,6	130
17		0	0	690	80,7	131
18		0	0	690	80,6	130
19		0	0	690	80,4	129
20		0	0	690	80,4	129

The effect of pressure, temperature and quantity of CO_2 on the RT output and the quadratic function of these process parameters, determined by equation (1), were tested. Using variational analysis (ANOVA), the values of various coefficients that determine the yield of RT were calculated.

The linear and quadratic effect of variables, as well as their interactions and coefficients, on the value of the variables obtained by ANOVA, a probable equation was calculated and derived, which represents the empirical interaction between the output of the RT and the independent variables (2).

$$\gamma = 0,70 + 0,033x_1 + 0,024x_2 + 0,073x_3 - 0,22022x_1^2 + 0,001668x_2^2 - 0,064x_3^2 + 0,007802x_1x_2 + 0,000088x_1x_3 + 0,0008878x_2x_3 \quad (2)$$

Where γ is the RT output (mgR / gdm) and x1 is the coded pressure value, x_2 is the coded temperature value and x_3 is the coded value of the amount of used SC-CO_2, as shown in Table 2.

Since R_2 (0.9088) is greater than 0.8, this indicates that the model well confirms the results.

Both linear and quadratic terms for the SC-CO_2 flow rate were more reliable ($p < 0.05$), as well as linear and quadratic pressure terms. The interactions between pressure, temperature and the amount of CO_2 were not significant. The RSM analysis proved the initial results and expectations, and that the pressure and flow rate of SC-CO_2, designated as x_1, x_3, x_{12}, x_{32} terms, were significant parameters of the model, which means that these independent variables (pressure and CO_2 flow) could be used to determine the yield of RT at SC-CO_2 extraction.

Despite the fact that the duration of extraction increases the yield of RT, it leads to a rise in the cost of such an operation and thus, a suitable time could be established based on the results of this study.

The effect of temperature on SC-CO_2extraction was observed through two possible effects on PT-extraction, although, as indicated by statistical analyzes, the effect of temperature on the yield of RT was insignificant. Knowing that SCFE is also designated as a "destruction" process, which means a combination of extraction and distillation, it is obvious that high pressure of SC-CO_2 was not favorable for extraction (high density), while high temperature increases the vapor pressure of various compounds. However, according to the molecular weight of RT, as well as other compounds that could be extracted from mulberry, a very slight effect of temperature on the yield of RT by extraction could be expected. Specifically, RT apparently has a low vapor pressure at the temperatures used in this study (30-50^{0}C) and the RSM analysis has proved that the temperature (as the encoded value of x_2) is an almost insignificant parameter that determines the yield of RT (GFR) by extraction (equation 1) . Moreover, increasing the temperature at constant pressure leads to a decrease in the density of CO_2 and thus reduces the strength of the solvent. Thus, the increase in the yield of RT with increasing temperature is also analyzed, but no noticeable effect is detected.

Bearing in mind that the more important independent changes with significant values and impact on the output of the RT are the pressure and flow rate of SC-CO_2, the following and simplified equation could be used to calculate the release of RT from mulberry:

$$\gamma = 0{,}70 + 0{,}32x_1 + 0{,}073x_3 - 0{,}22x_1^2 - 0{,}064x_3^2$$

These equations can be used to yield (γ) RT at pressures from 16.9 to 32.2 MPa (coded value x1 from -1.662 to +1.668) and for SC-CO2 flow from 11.08 to 28.84 (also used as $x_2$coded value from -1.688 to +1.668).

Comparison of the extracted amount of RT calculated using equations (2) and (3) and experimentally found values showed only a small difference in the correlation coefficient between the calculated and experimentally determined values of γ (r = 0.987 and using equation (2), r = 0.989) to show the results of the RSM-CCRD analysis [8,9].

RESULTS AND ITS DISCUSSION

It was shown that the amount of extract that could be obtained from mulberry varies from 0,52 to 1,6%. These results can be compared with those published data, where the extraction was combined with microwave treatment and they amounted to 8.7% -16.8%. Some other literature data refer to SC-CO_2 extraction [4]. However, these authors use the term defined as the ratio of SC-CO_2 output to that which occurs in classical extraction methods. They found that SC-CO2 extraction yields of 20-85%, while in the classical method the yield fluctuates between 11- 43%.

According to the RSM analysis, the optimal conditions for the maximum yield of RT (0,52 mg RT / gdm) are 15.8 MPa, 30.5 ° C and 20.08 gCO_2 / gdm. (The amount of CO_2 used or for 150 minutes of extraction).The CO_2 density calculated for the optimum pressure and temperature values is 725 kg / m^3, which was also found as a result of a preliminary correlation analysis between the yield of RT and the density of CO_2. As shown in the literature, the optimum conditions for SC-CO2 extraction are 15.8 MPa, 30.5 ° C and 129.8 min [4,5], which are quite close to the optimum conditions found by us.

The results of SC-CO_2 optimization carried out in this study as well as those available in the literature for various plants have proved that the extraction temperature has little or no influence on the extraction (yield) of RT. It is also important to note that this study for the first time provides data on SC-CO_2 extraction of RT from mulberry berries.

CONCLUSION

In this study, RSM in combination with CCRD is adequately used to describe and predict the yield of RT under different conditions of SC-CO_2 extraction. It is established that the yield of RT is mainly dependent on the pressure and amount of SC-CO_2 used for extraction. It turned out that there is a significant dependence on the linear and quadratic terms for the relationship between the yield of RT and these parameters. There was no significant interaction between the three parameters of the process (pressure, temperature, and amount of SC-CO_2). The optimal conditions were determined: 15.8 KPa, 30.5^{0}C and 20.08 g CO_2 / gdm. Consumption of CO_2 for extraction of RT from berries of mulberry, using SC-CO_2; The maximum yield of RT was 0,052 mg from 1 g of dry material (about 0.5% of the total extract) can be carried out under certain optimum extraction conditions; it will be possible to obtain the maximum yield of RT at a density of SC-CO_2 equal to approximately 725 kg / m^3.

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