BETANINE EXTRACTION FROM BETA VULGARIS – EXPERIMENTAL RESEARCH AND STATISTICAL MODELING

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Sfecla roşie (Beta vulgaris) constituie sursa principală de colorant natural roşu, extractul obținut din aceasta fiind catalogat ca roşu de sfeclă şi indexat ca E162 (Directive 95/45/EC). Principalul component al acestui extract este betanina. In această lucrare s-a studiat eficiența unor soluții apoase utilizate pentru extracția betaninei din sfeclă roşie. Parametrii urmăriți au fost concentrația betaninei în extracte şi stabilitatea extractelor obținute. De asemenea, a fost elaborat un model statistic, necesar pentru optimizarea condițiilor de extracție.

Beetroot (Beta vulgaris) is the main source for the natural red dye, the extracted obtained being catalogued as "beetroot red", which is known as E162 (Directive 95/45/EC). The main component of this extract is betanine. In this paper, the efficiency of different aqueous solutions used from betanine extraction from beetroot was studied. The control parameters were betanine concentration in the extracts and the stability of the obtained extracts. A statistical model was developed for optimizing extraction conditions.

Keywords: extraction, betanine, beetroot, statistical modelling

1. Introduction

Betanines or betalains are natural dyes extracted from different fruits and vegetables. They are largely used as food colorants in food products like yogurts, ice cream and other products [1-3]. Recent studies have shown that betanines have antioxidant, antimicrobial and antiviral activity [4].

Beetroot (Beta vulgaris) is the main source of natural red dye, known as "beetroot red". Betanine is the main component of the red colorant extracted from Beta vulgaris. Immediately after extraction, betanine is exposed to degradation.

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The pigment stability is influenced by factors such as enzymes, temperature, oxygen and pH [4, 5].

Besides betanine, another pigment which is extracted from beetroot is vulgaxanthine.

Betanine content in beetroot varies from 100 mg/100 g fresh product to 16 – 38 mg/100 g dried vegetable product. Therefore, the plant species and harvesting conditions are important factors to be considered when selecting the suitable raw material [6].

Betanine can be obtained from beetroot by milling followed by pressing, filtration and evaporation of the resulted juice [7]. The product of this process is a red powder. Solid liquid extraction, carried out under conditions which lead to a maximum extraction yield and minimum pigment degradation, continues to be a useful method for obtaining beetroot juice.

In this paper, the extraction capacity of weak acid solutions and hydroalcoholic solutions was tested. Betanine concentration in the extracts and stability of solutions with highest betanine content were studied.

A factorial study was also carried out in order to asses the influence of operating parameters – temperature, solid/liquid ratio and pH, on the process's performance.

2. Materials and methods

2.1 Extraction procedure

Commercially available beetroot was used as extraction material. The vegetable material was cut into slices having approximately 21 mm length, 5 mm width and 1-2 mm height. The following solvents were used in the first series of experiments: 1 – distilled water and the following aqueous solutions: 2 –citric acid solution 1%, 3 –citric acid solution 0.5%, 4 –citric acid solution 0.2%, 5 – ascorbic acid solution 0.1%, 6 – ethanol solution 50%, 7 – ethanol solution 20%, 8 –citric acid 0.5% and ascorbic acid 0.1% solution, 9 –citric acid 0.2% and ascorbic acid 0.1% solution, 10 – ethanol 20% and citric acid 1% solution and 11 –ethanol 20% and citric acid 0.5% solution. Batch extraction were carried out at 25 ^{o}C, liquid solid / ratio of 5 : 1, extraction time 3 minutes.

2.2 Stability studies

The extraction solutions with the highest betanine content were stored for 10 days at room temperature. The betanine content was monitored during this period.

2.3 Analysis of betanine content

Betanine content in the extracts was determined spectrophotometrically, using an UV/VIS Cintra 6 spectrophotometer (GBS Australia). Betanine has an absorption maximum at 535 – 540 nm, but absorbs also at 476-478 nm, where vulgaxanthine has a maximum. The quantity of betanine extracted from 1 g of vegetable material was calculated using the following equation:

$$m_i = \frac{A_i \cdot F_d \cdot \overline{M}}{\varepsilon \cdot l} \cdot \frac{V_e}{1000 \cdot m_s} \tag{1}$$

The significance of the terms used is as follows:

m_i - amount of extracted betanine per g of raw material used;

A_i - sample absorption;

$\overline{M}$ - average molecular mass, in g/mol;

ε - molar extinction coeffient (1120 $L \cdot cm^{-1} \cdot mol^{-1}$ for betanine) [5];

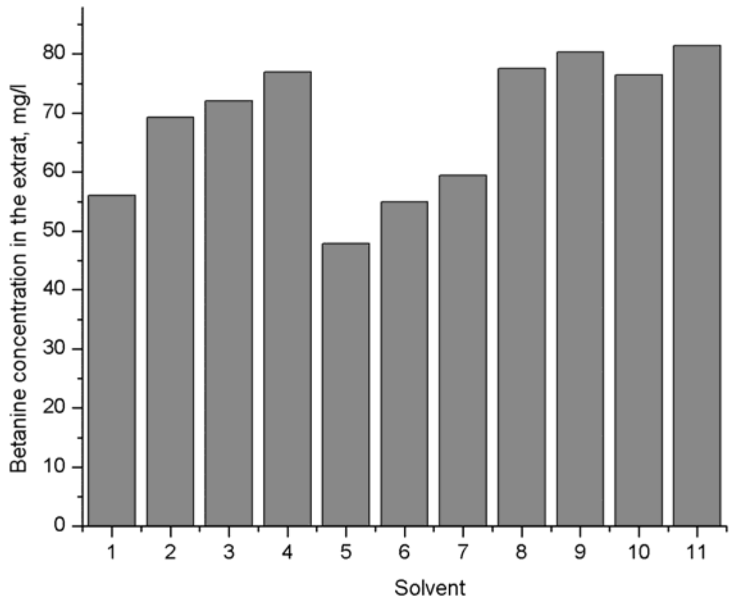
V_e extract volume, in mL;

m_s - mass of vegetable solid used for extraction.

F_d - dilution factor

3. Experimental results and discussion

The experimental results are graphically presented in figures 1 and 2. Figure 1 presents betanine concentration in the extracts after 3 minute of extraction.



Fig, 1. Betanine concentration in primary extracts
1 – distilled water; aqueous solutions: 2 – citric acid solution 1%, 3 – citric acid solution 0.5%, 4 – citric acid solution 0.2%, 5 – ascorbic acid solution 0.1%, 6 – ethanol solution 50%, 7 – ethanol solution 20%, 8 –solution citric acid 0.5% and ascorbic acid 0.1%, 9 - solution citric acid 0.2% and ascorbic acid 0.1%, 10 – solution ethanol 20% and citric acid 1%, 11 – solution ethanol 20% and citric acid 0.5%

One can easily notice that the solvents with the highest extraction capacity from this study are: aqueous solution of citric acid 0.2% and ascorbic acid 0.1% solution (position 9 from fig. 1) and aqueous solution of ethanol 20% and citric acid 0.5% (position 11 from fig.1).

Figure 2 presents the variation of betanine content in the two extracts, during 10 days storage. It is clear that the extract in aqueous solution of citric acid 0.2% and ascorbic acid 0.1% was much more stable.

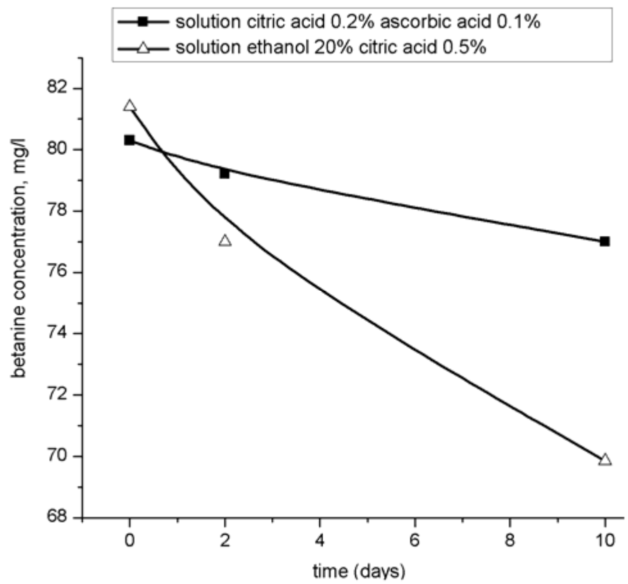


Fig. 2.Variation of betanine concentration in the stored extracts at room temperature

4. Factorial experimental design

Considering that the main factors influencing the extraction process performance and betanine stability are temperature, pH and solid / liquid ratio, the experimental study was carried out according to a 2^3 factorial experimental program. Table 1 presents the variation levels of the 3 parameters, while table 2 presents the experimental matrix.

Table 1

Variation levels for process factors

Process factors	Inferior level	Superior level
Temperature (^oC)	20	70
Liquid/solid ratio	5	10
pH	2.5	8

Table 2

Experimental matrix

Experiment	T, ^oC	$R_{L/S}$	pH
O	20	5	2.5
1	70	5	2.5
2	20	10	2.5
3	20	5	8
12	70	10	2.5
13	70	5	8
23	20	10	8
123	70	10	8

The results of these experiments expressed as mg extracted betanine/g beetroot are graphically presented in figures 3 and 4.

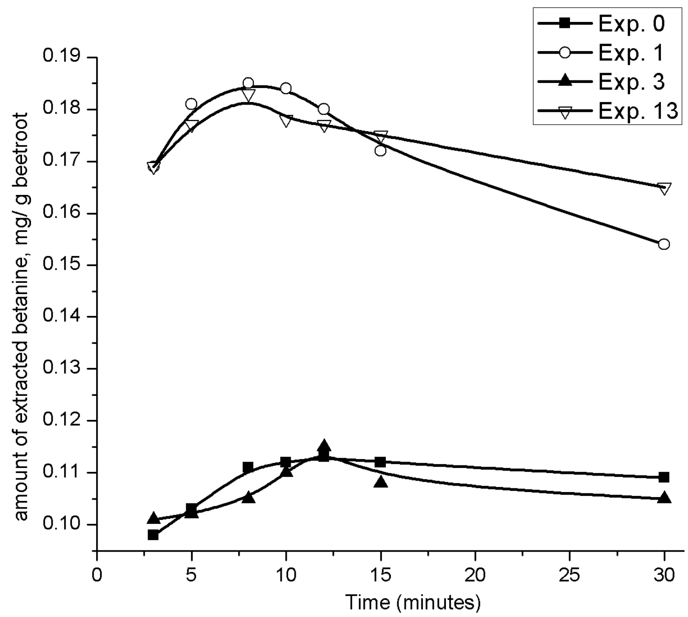


Fig. 3.Variation of amount of extracted betanine versus temperature and pH

Figure 3 presents the experimental results obtained when the liquid/solid ratio was 5/1. Comparing experiment 0 with experiment 1, and experiment 3 with experiment 13 one can notice that the temperature has a positive influence on betanine extraction yield, beat leads to target compound decomposition after only 10 minutes. Also, comparing the results of experiment 1 with the results of experiment 13 it can be seen that, at high temperature, the pH doesn't have a significant influence on process performance; on the other side, at low temperature, the acid medium has a positive influence (experiments 0 and 1).

Figure 4 presents the results of experiments carried out at the two levels of temperature and liquid/solid ratio, keeping the pH value constant at 2.5.

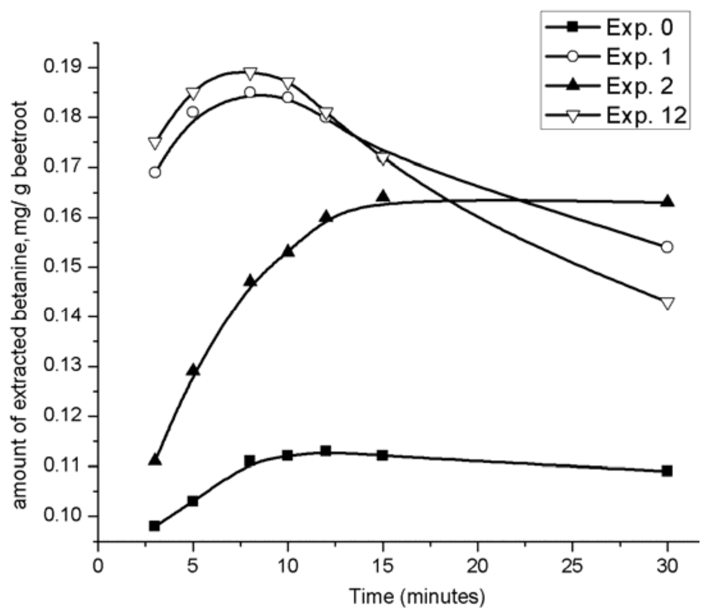


Fig. 4.Variation of amount of extracted betanine versus temperature and L/S ratio

The above figure shows that these two factors have a positive influence on the extraction dynamic and on the amount of extracted betanine.

5. Statistical modeling of the extraction process

The factorial experimental program allowed us to establish a statistical model which gives the relation between the process response and the process factors. Based on the experimental results presented above the amount of extracted betanine in mg/g beetroot used for extraction after 10 minutes, was chosen as dependant variable (response factor). Table 3 presents the process response as a function of process factors, expressed with dimensional and non-dimensional values. Non-dimensional values were calculated using the following relations:

$$z_j^0 = \frac{z_j^{\min} + z_j^{\max}}{2} \qquad \Delta z_j = \frac{z_j^{\max} - z_j^{\min}}{2} \qquad x_{ji} = \frac{z_{ji} - z_j^o}{\Delta z_j} \tag{2}$$

where:

z_{ji} - dimensional value of process' factors, j = 1…3, i – experiment number

z_j^0 - central value of each factor

x_{ji} - non-dimensional value of each factor, j = 1…3, i – experiment number.

Table 3

Matrix of experimental results using 2^3 factorial design

Dimensional factors' values				Non-dimensional factors' values			Process response's values
Exp. No	z_{1i} (temperature, °C)	z_{2i} (liquid/solid ratio)	z_{3i} (pH)	x_{1i}	x_{2i}	x_{3i}	y_i
0	20	5	2.5	-1	-1	-1	0.112
1	70	5	2.5	+1	-1	-1	0.184
2	20	10	2.5	-1	+1	-1	0.153
3	20	5	8	-1	-1	+1	0.11
12	70	10	2.5	+1	+1	-1	0.187
13	70	5	8	+1	-1	+1	0.178
23	20	10	8	-1	+1	+1	0.126
123	70	10	8	+1	+1	+1	0.185

The following regressin equation, which gives also the effects of factors' interferences, was proposed for this experimental plan:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \beta_{123} x_1 x_2 x_3 \quad (3)$$

Due to ortogonal behavior of experimental matrix ([9]) the coefficients β_k, $k = 0,1,2,3$, β_{kl}, $k = 1,2; l = 2,3; k \neq l$ and $\beta_{klm}, k = 1, l = 2, m = 3$ were calculated using relation (4):

$$\beta_k = \frac{\sum_{i=1}^{N} x_{ki} y_i}{N}; \; \beta_{kl} = \frac{\sum_{i=1}^{N} x_{ki} x_{li} y_i}{N}; \; \beta_{klm} = \frac{\sum_{i=1}^{N} x_{ki} x_{li} x_{mi} y_i}{N} \quad (4)$$

with N=8.

Using the experimental data, linear regression leads to the following relation:

$$y = 0.154 + 0.029 x_1 + 8.375 \cdot 10^{-3} x_2 - 4.63 \cdot 10^{-3} x_3 - 5.88 \cdot 10^{-3} x_1 x_2 + 2.6 \cdot 10^{-3} x_1 x_3 - 2.63 \cdot 10^{-3} x_2 x_3 + 3.625 \cdot 10^{-3} x_1 x_2 x_3 \quad (5)$$

Student test method was used for testing the significance of the above equation coefficients, considering a confidence level 1-α = 0.95. For this purpose, the process' response in the central point of the experiment was determined [9]. Table 4 presents the matrix of experimental results when factors have central values.

Table 4

Matrix of experimental results when factors have central values

Dimensional factors' values				Non-dimensional factors' values			Process response's values
Exp. No	z_{1i} (temperature, °C)	z_{2i} (liquid/solid ratio)	z_{3i} (pH)	x_{1i}	x_{2i}	x_{3i}	y_i
1	45	7.5	5.25	0	0	0	0.165
2	45	7.5	5.25	0	0	0	0.170
3	45	7.5	5.25	0	0	0	0.170

With the above data, the variances square roots needed for testing the coefficient significance were calculated (equations 6 and 7). The obtained results were further used for calculating the Student variable, t_j, for each coefficient (equation 8). The calculated t_j values were compared with "t" value corresponding to accepted significance level [10].

$$\bar{y}_c = \frac{\sum_{i=1}^{3} y_i}{3} = 0.1683 \qquad s_{rp} = \sqrt{\frac{\sum_{i=1}^{3} (y_i - \bar{y}_c)^2}{2}} = 0.002887 \qquad (6)$$

$$N = 8 \qquad s_{\beta j} = s_{\beta jl} = s_{\beta jkl} = \frac{s_{rp}}{\sqrt{N}} = 0.00121 \qquad (7)$$

$$t_j = \frac{|\beta_j|}{s_{\beta j}} \qquad (8)$$

Table 5 presents the results of calculations related to the significance of β coefficients.

Table 5

Testing the coefficients' sinificance

No.	Hypothesis	Values of student tests' variables, t_j	$t_{\alpha/2}$ for ν=2 and 1-α=0.95	$t_j : t_{\alpha/2}$	Conclusion on the hypothesis
1	$\beta_o=0$	$t_0=151.25$	4.3	$t_0>t_{\alpha/2}$	rejected
2	$\beta_1=0$	$t_1=28.5$	4.3	$t_1>t_{\alpha/2}$	rejected
3	$\beta_2=0$	$t_2=8.2$	4.3	$t_2>t_{\alpha/2}$	rejected
4	$\beta_3=0$	$t_3=4.53$	4.3	$t_3>t_{\alpha/2}$	rejected
5	$\beta_{12}=0$	$t_{12}=5.75$	4.3	$t_{12}>t_{\alpha/2}$	rejected
6	$\beta_{13}=0$	$t_{13}=2.57$	4.3	$t_{13}<t_{\alpha/2}$	accepted
7	$\beta_{23}=0$	$t_{23}=2.57$	4.3	$t_{23}<t_{\alpha/2}$	accepted
8	$\beta_{123}=0$	$t_{123}=3.55$	4.3	$t_{123}>t_{\alpha/2}$	accepted

Thus, the final equation of the statistical model is:

$$y = 0.154 + 0.029x_1 + 8.375 \cdot 10^{-3} x_2 - 4.63 \cdot 10^{-3} x_3 - 5.88 \cdot 10^{-3} x_1 x_2 \quad (9)$$

The above equation shows that all three factors influence the performance of betanine extraction process. From the values of coefficients one can conclude that temperature has the highest influence on betanine extraction yield. The last member of the equation is referring to the interferences between temperature and liquid/solid ratio.

The last step of statistical modeling consists in testing the model significance, in other words validation of the obtained equation, using the Fisher test. For this purpose, the residual variance was calculated, using the following equation:

$$s_{rz}^2 = \frac{\sum_{i=1}^{N} (y_i - y_i^c)^2}{N - n_\beta} \quad (10)$$

$$s_{rz}^2 = 7.2 \cdot 10^{-5}$$

where:

y_i^c - calculated values of process' responses

y_i - process responses' value experimentally determined

n_β - number of coefficients of the statistical model, in this case 5.

The residual variance was calculated using equation 9 for determination of calculated values of process' responses and the experimental data reported in table

3. The value of F variable, associated to Fischer test was calculated using equation 11:

$$F = \frac{s_{rz}^2}{s_{rp}^2} = \frac{7.22 \cdot 10^{-5}}{8.33 \cdot 10^{-6}} = 8.66 \qquad (11)$$

In order to asses the validity of the developed statistical model, the calculated value of F must me compared to the tabular value corresponding to the chosen significance level. In this case, the tabular value of F for a significance level $\alpha = 0.5, \nu_1 = 3; \nu_2 = 2$, where ν_1 and ν_2 represent the number of degree of freedom corresponding to nominator variance and denominator variance respectively, is $F_{3,2,0.05} = 19.16$ [11]. Comparing the theoretical value to the calculated value for F, one can notice that:

$$F \prec F_{3,2,0.05} \qquad (12)$$

This means that the statistical model given by equation (9) is adequate for describing the dependence of process response to the process' factors for the studied range.

6. Conclusion

Week acid solutions were tested for use as solvents for betanine extraction from dried beetroot. The experimental study showed that aqueous solution of citric acid 0.2% and ascorbic acid 0.1% and aqueous solution of ethanol 20% and ascorbic acid 0.5% are best suited for this purpose. However, even if the extraction yield when using these solvents is good, another factor which should be considered when choosing the extraction solvent is betanine stability in the resulted extract.

The experimental results showed that, after 10 minutes, practically the extraction process reaches its maximum performance.

A factorial experimental program was also developed. Experimental results proved that extraction performance is influenced by temperature, liquid/solid ration and pH of extraction solution. The experimental results were used for developing a statistical model, which gives the correlation between the amount of extracted betanine and the factors influencing the process. The coefficients of the obtained equation show that temperature has the highest influence on the extraction yield. Fisher test applied to the developed statistical model showed that this is fit for describing the dependence of process response to the process' factors for the studied range.

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