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EXPERIMENTAL DESIGN FOR ENHANCEMENT OF BETANIN IN BEET JUICE USING PULSED ELECTRIC FIELD

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Abstract: *The influence of strength and pulses number of Pulsed Electric Field (PEF) treatment on juice extraction and betanin concentration in has been investigated in our studies. Pulsed electric field (PEF) treatment is an innovative and promising method for non-thermal processing of foodstuff. It is a good alternative to conventional cell membrane permeabilization methods such as thermal treatments and the addition of chemicals as well as of enzymes. The principal effect resulting from pulsed electric field (PEF) interaction with the biological cell is the phenomenon of eletroporation which is a creation of pores on membrane cells in order to increase the permeability. The influence of strength and pulses number of pulsed electric field (PEF) treatment for enhancing betanin extraction from beet food has been investigated in this work. This paper is aimed to analyze the effectiveness of the PEF pretreatment of beet juice extraction process based on Response Surface Modeling (RSM) for identifying the set point of the betanin extraction process using pulsed electric field pre-treatment. The experiments were carried out on a laboratory experimental bench. Obtained results pointed out that investigated parameters of PEF treatment have significant effect not only in juice yield, but also for enhancement of the betanin concentration of final product.*

Key words: *Pulsed electrical field (PEF), Electroporation, Betanin, Yield juice, Membrane cell, Food.*

1. Introduction

Betanin is a dynamic red pigment primarily responsible for the characteristic colour of ripe fruits beet. He is recently attracting attention of the food industry due to its wide use as a food additive. Betanin degrades when subjected to light, heat, and oxygen [11].

The betanin content in beet fruit may be enhanced by improved management techniques such as variety selection, growth conditions, harvest time, etc. Irrespective of the strategies used to increase betanin concentration in a fruit, it is critical to establish an effective means of

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extracting maximum amount of betanin from the fruit. Various technologies, such as membrane separation technology, supercritical fluid CO_2 extraction technology [18], microwave extraction [4] and solvent extraction [7] have been used for pigments extraction.

The possibility of using pulsed electric field to rupture the cell membrane of plant materials was discovered from the 1960's for 1970's [3], [21]. Since then it has been used in pasteurizing juice and liquid food products [2], [10]. The technology has been found effective in enhancing juice extraction from apple, sugar beet, and alfalfa [9], [12], [16]. Plant cell cultures producing secondary metabolites are permeabilized by electroporation by moderate high voltage pulsed electric field (1-5 kV/cm) [1].

The betanin content in beet fruit may be enhanced by using pulsed electric field pretreatment and mechanical extraction of juice beet process was invastegated.

The PEF-treated extraction process depends on a multitude of factors. In such application, the list of factors influencing the process includes the pulse duration, the electric field, the number of pulses [13], [14], [17], and the average power and so on…. Thus, it's not simple to determine with precision the optimal values of the process factors. An experimental procedure for optimizing the extraction process was employed using a home-made experimental set-up, comprising a pulse generator, a treatment chamber and a pressing machine. Three "one-factor-at-a-time" experiments, corresponding to three controllable factors, followed by a factorial design were performed based on a two steps strategy: fixing the variation domain of the input variables and searching the optimum set point.

2. Pulsed Electric Field For Electroporation

Exposing a biological cell (plant, animal and microbial) to a high intensity electric field (kV/cm) using very short pulses (µs to ms) induce the formation of temporary or permanent pores on the cell membrane (Figure 1).

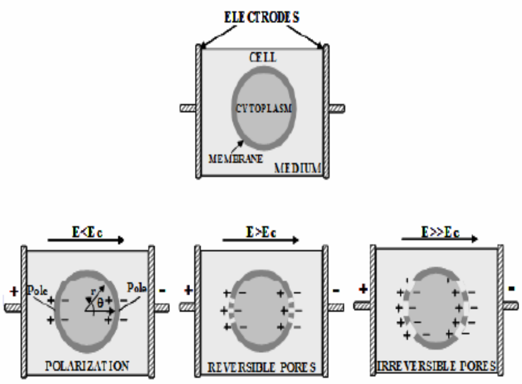


Fig. 1. *Schematic diagram of a cell exposed to electric field (electroporation process)*

This phenomenon, called electroporation, causes the permeabilization of cell membrane i.e. an increase of its permeability and if the intensity of the treatment is sufficiently high, cell membrane disintegration occurs. The mechanism of electroporation is not yet fully understood. Several models have been suggested to explain this complex phenomenon.

The electroporation for permeabilization of the cell membrane is used in many fields such as biotechnology, cell biology, medicine and food industry. Mass transfer processes such as solid-liquid extraction and drying as well as food preservation are important unit operations of the food industry requiring the electroporation of the cell membrane.

3. Materials and Methods

Fresh beets were obtained at local market of fruits and vegetables. After sorting and cleaning operations, they were comminuted with a domestic food processor (Thomson, THMX05736 Model) for 5 min to obtain a homogenous mash. The obtained mash was then kept in a closed vessel to prevent evaporation prior to use.

The treatment chamber consisted in an insulated cylinder made of plastic (Teflon, PTFE) of length 140 mm and diameter 70 mm (Figure 2). The electrodes are made of a cylindrical plunger and a disc base of a same diameter 70 mm having a rigid structure for juice pressing operation, both made with stainless steel. Extracted juice was filtered through a stainless steel sieve placed on top of the perforated plunger. Juice extracted during pressing was collected in a plastic collector placed under the treatment chamber. The volume of the treatment chamber was 192.3 ml (Figure 3). For all experiments, the same treatment chamber was used for both pressing and pulsed electric field treatment steps.

The pressure was applied using a hydraulic pressing machine (Mega, 15 tons). Just after PEF treatment, the filled treatment chamber was pressed until a defined pressure of 100 kg/cm^2, and was then held at this pressure for 5 min. For all experiments, the thickness of the sample was equal to 2 cm, corresponding to a sample mass of 60 g.

An electronic balance of 0.1 g precision was used to weight the beet juice collected in flacon tubes. During a PEF treatment, the food material is placed between two electrodes forming a treatment chamber and high voltage repetitive pulses are applied across the system in order to achieve membrane breakdown.

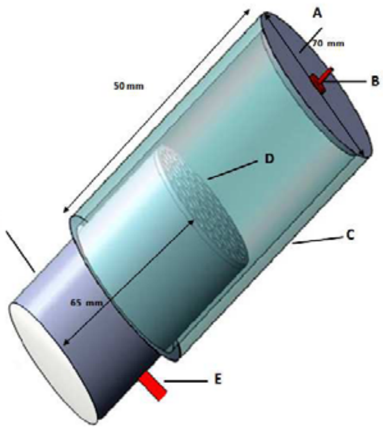


Fig. 2. *Schematic description of the treatment chamber (All dimensions are in mm): (A) Stainless Steel disk(upper electrode), (B) High voltage connection, (C) Teflon cylinder, (D) Stainless steel sieve, (E) Ground connection, (F) Perforated stainless steel plunger (lower electrode)*

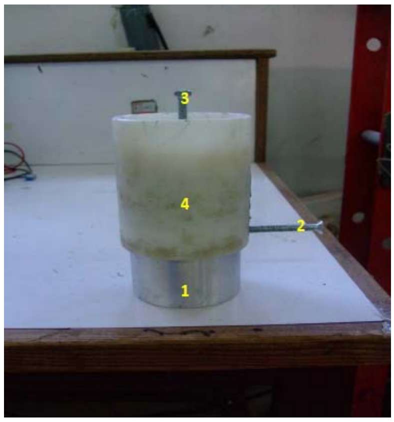


Fig. 3. *Treatment chamber used in the experiments: 1 – perforated stainless steel plunger (lower electrode), 2 – ground connection, 3 – high voltage connection, 4 – Teflon cylinder*

3.1. High Pulsed Electric Field Treatment

The high voltage pulse generator provides electrical pulses of the desired voltage, shape and duration. The DC power supply charges the capacitors bank to the determined voltage. Using this device, the AC power from the utility line 50 Hz, is converted in alternating current (AC) high voltage power and then rectified to a DC high voltage power. The energy provided by the DC power supply is temporarily stored in the capacitor(s) and then delivered very quickly in form of pulses, to the food to generate the necessary electric field strength.

For all experiments, the same treatment chamber was used for both pressing and pulsed electric field treatment tests. Pulsed electrical field treatment of the beet mash was achieved by using a PEF generator, represented in Figure 4.

Fig. 4. *The experimental set-up: Auto-transformer - (2) HV DC power supply - (3) Electrostatic voltmeter - (4) HV measuring probe - (5) Charging resistors - (6) HV charging capacitor - (7) spark-gap discharger - (8) Treatment chamber*

A variable autotransformer (AT) (LangloisALT5A) was used to supply a Direct Current high voltage (HV) power supply in order to control the HV output. A 100-Ω resistor was used to limit the current passing through the capacitors used to store the energy. The treatment voltage applied to the treatment chamber is related to the distance between the 15-mm diameter stainless steel spheres of the spark-gap discharger.

3.2. Preliminary Results of Juice Extraction with PEF Treatment

PEF pre-treatment of beet tissue is followed by the application of a pressure of 100 kg/cm^2, at ambient temperature, for a duration of 5 min. PEF treatment resulted in a significant increase in the yield of juice. More juice was extracted from the treated mash as shown in Figure 5. Obtained results represented in Table 1 show that pulsed electric field treatment increases the quantity of extracted beet juice by more than 90% compared with untreated one.

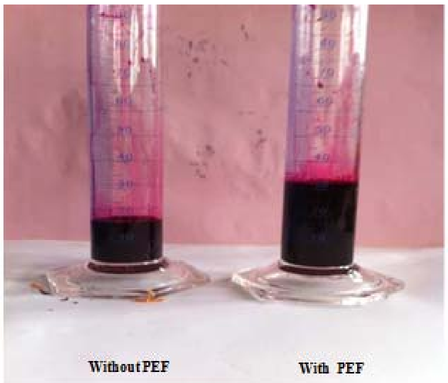


Fig. 5. *PEF treated and Nn-treated juice* extraction

Table 1. *Obtained results of beet juice extraction*

Samples of beet	Treatment parameters	Mass of extracted juice
PEF-treated sample	3 kV/cm, 100 pulses, 20 μs	36.6 g
Untreated sample	100 kg/ cm^2	19.3 g

The increase of the extracted juice is due to the new cellular structure of the plant as a result of the electroporation of the cell membrane because of an interaction with the pulsed electric field.

3.3. Design of PEF Treated Juice Extraction Experiments

Methodology of the experimental designs makes it possible to determine the number of experiments to be achieved according to a well defined objective, to study several factors simultaneously, to reduce dispersion related to measurements, to appreciate the effects of coupling between factors and finally to evaluate the respective influence of the factors and their interactions [6], [8], [19].

The Composite Centred Faces design (CCF), which gives quadratic models, was adopted. A quadratic dependence is established between the output function to optimize (response) and the input variables [15], [20].

Classical "one-factor-at-a-time" experiments are carried out to identify the domain of variation of the three following factors:

1. Electric field level *E* (kV/cm);
2. Number of pulses *n*;
3. Pulse duration T (µs).

The pulse duration was varied by using appropriate values of the charging capacitor. As the voltage wave shape is bi-exponential, the pulse duration *T* corresponds to 37 % of the amplitude (Figure 6).

Thus, following values were obtained: T = 20 µs for C = 1 µF, T = 40 µs for C = 2 µF and T = 60 µs for C = 3 µF.

Before starting the experiments, it is necessary to set the best and suitable design which can the process with the most possible precision. In this paper, the Composite Centred Faces design (CCF), which gives quadratic models, was adopted. It is possible to determine a quadratic dependence between the output function to optimize (response) and the input variables u_i *(i = 1,.......,* (factors):

$$y = f(u_i)c_0 + \sum c_i u_i + \sum c_{ij} u_{ij} + \sum c_{ii} u_i^2 \quad (1)$$

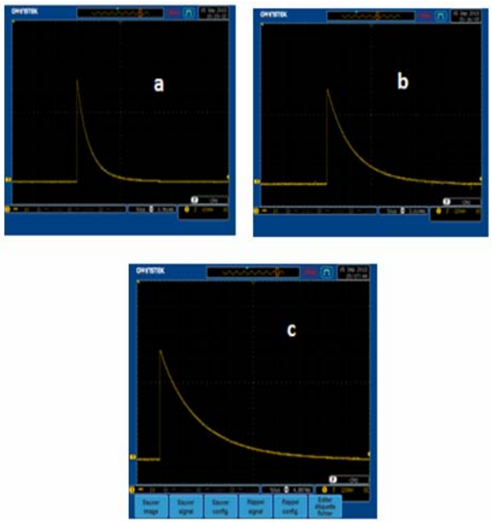


Fig. 6. *Current wave forms delivered by the pulse generator for different value of capacitance C: (a) T = 20 µs (C = 1 µF), (b) T = 40 µs (C = 2 µF), (c) T = 60 µs (C = 3 µF)*

Knowing that Δu_i and Δu_{io} are respectively the step of variation and the central value of factor *i* i, reduced centred values of input factors may be defined by the following relation:

$$x_i = (u_i - u_{i0}) / \Delta u_i \qquad (2)$$

With these new variables, the output function becomes:

$$y = f(x_i) = a_0 + \sum a_i x_i + \sum a_{ij} x_{ij} + \sum a_{ii} x_i^2 \quad (3)$$

The coefficients can be calculated or estimated by a data-processing program, in such a way to have a minimum variance between the predictive mathematical model and the experimental results.

MODDE 5.0 software (Umetrics AB, Umea, Sweden) was used, which is a Windows program for the creation and the evaluation of experimental designs [22].

The program assists the user for interpretation of the results and prediction of the responses. It calculates the coefficients of the mathematical model and identifies best adjustments of the factors for optimizing the process. Moreover, the program calculates two significant statistical criteria which make it possible to validate or not the mathematical model, symbolized by R^2 and Q^2.

The former is called the goodness of fit, and is a measure of how well the model can be made to fit the raw data; it varies between 0 and 1, where 1 indicates a perfect model and 0 no model at all. The latter is called goodness of prediction, and estimates the predictive power of the model. Like R^2, Q^2 has the upper bound 1, but its lower limit is minus infinity. For a model to pass the diagnostic test, both parameters should be high, and preferably not separated by more than 0.2–0 .3.

4. Results and Discussion

The experimental procedure to obtain a mathematical model starts with following “one-factor-at-a-time” experiments.

Experiment 1: Variable pulsed electric field intensity E (1- 4 kV/cm), at constant values of n = 100 pulses and T = 20 µs.

Experiment 2: Variable number of pulses n (50-200), at constant values of E = 3 kV/cm and T = 20 µs.

Experiment 3: Variable pulse duration T (20-60µs) of the pulse generator at constant values of E = 3 kV/cm and n = 100 pulses.

Obtain results in this section served to the definition of the domain of variation of E, n and T.

The results of Experiments 1–3 are given, respectively, in Figures 7–9. The evaluation of the process was estimated by the mass of extracted juice which was represented according to the three control factors in Figures 7–9.

Thus, the graph in Figure 7 shows that in the conditions of Experiment 1, the mass of extracted juice increases up to 3 kV/cm, and then decreases for higher values of *E*. Thus, $E_{min} = 1$ kV/cm and $E_{max} = 3$ kV/cm were retained as the limit values for the electric field.

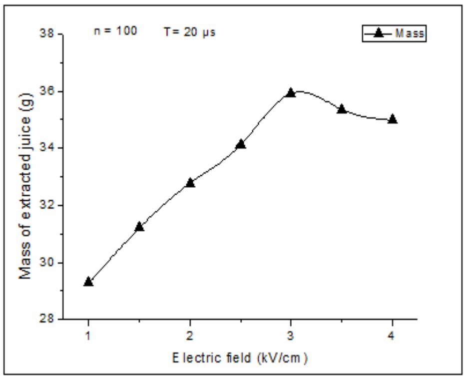


Fig. 7. *Evolution of extracted juice mass according to the electric field (n=100, T= 20 µs)*

In the conditions of Experiment 2 (Figure 8), we noticed the same variation concerning the influence of of pulses number. The mass of extracted juice firstly increased with the pulses number up to n =100 pulses, then it decreased. Consequently, the domain of variation of this factor was defined as $n_{min} = 50$ and $n_{max} = 150$ pulses.

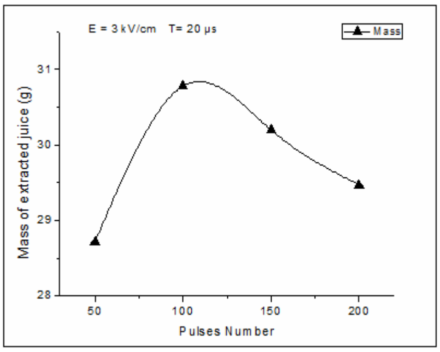


Fig. 8. *Evolution of extracted beet juice mass according to the pulses number (E= 3 kV/cm, T = 20 µs)*

Furthermore, results of experiments 3 (Figure 9) obtained according to the pulse duration τ show that τ should not exceed 55 µs.

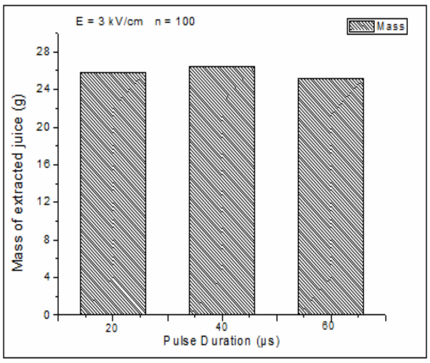


Fig. 9. *Evolution of extracted beet juice mass according to the pulse duration (E= 3 kV/cm, n = 100)*

Otherwise the mass of the juice will decrease, causing the diminution of the extraction efficiency. Indeed, when the pulse duration is higher the beet cells receive a great amount of energy, causing the reverse effect. So, we opted for the T_{min} = 30 µs and T_{max} = 80 µs as limits of variation domain of T.

The identification of the set point (E_0, n_0 and T_0)by using a central CCF design was performed; the two levels "max" and "min" are the limits established in previous section for each of the four control variables (E_{min}, E_{max}), (n_{min}, n_{max}) and (T_{min}, T_{max}), the central point (E_c, n_c and T_c) being calculated as follows:

$$Ec = (E\min + E\max)/2 = (1+3)/2 = 2kV/cm \quad (4)$$

$$nc = (n\min + n\max)/2 = (50+150)/2 = 100Pulses \quad (5)$$

$$Tc = (T\min + T\max)/2 = (20+60)/2 = 40\mu s \quad (6)$$

The results of all the experiments are given in Table 2.

Table 2. *Results juice mass experience extract according to variation in treatment values*

Exp. N°	*E* [kV/cm]	*T* [µs]	*n*	*Masse of juice* [g]
1	1	20	50	29,02
2	3	20	50	35,07
3	1	60	50	29,6
4	3	60	50	32,38
5	1	20	150	30,82
6	3	20	150	36,2
7	1	60	150	30,83
8	3	60	150	32,67
9	1	40	100	31,55
10	3	40	100	36,15
11	2	20	100	32,79
12	2	60	100	32,68
13	2	40	50	33,19
14	2	40	150	34,68
15	2	40	100	34,74
16	2	40	100	34,74
17	2	40	100	34,74

According to all of the experiments modeling software MODDE 5.0 gave us a mathematical model of juice extraction using pulsed electric field treatment. This mathematical model is very satisfactory because the coefficients R^2 and Q^2 are very close to 1 (Figure 10). MODDE 5.0 also gives the effect of each parameter on extracted juice yield (Figure 11).

The mass of extracted juice M is the response of the experimental design, the mathematical model was obtained:

$$M = 34.05 + 2.06E + 0.59n - 0.57T - 0.48E^2 - 0.4n^2 - 1.6T^2 - 0.85E*T - 0.18T*n \quad (7)$$

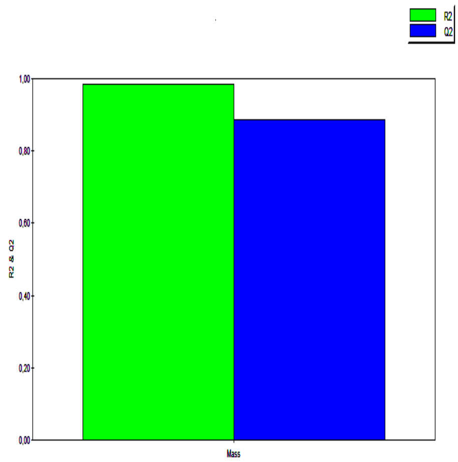


Fig. 10. *Representation of descriptive quality and predictive quality of mathematical model of juice extraction*

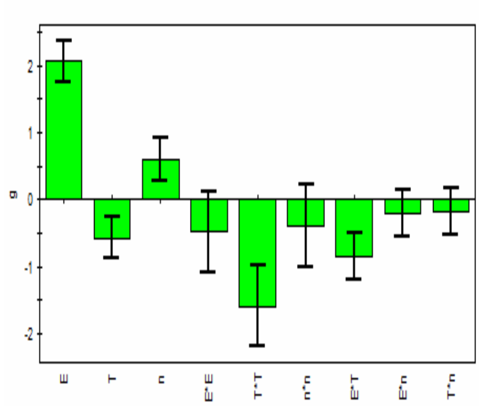


Fig. 11. *Plotted coefficients of the obtained model*

According to the mathematical model, the outcome of the process, i. e. the mass of the extracted juice, should be higher by increasing both the electric field *E* and the pulses number and decreasing the pulse duration, the most significant factor being *E*. Moreover, except the interaction between the electric field and the pulse duration, other interactions are not significant. This means that the delivered energy during one pulse has an important effect on the electroporation process.

Figure 12 shows the iso-response contours obtained with the present model, for n = 100. In addition, the software offers the possibility to identify the optimal values of the factors which should give the highest amount of extracted juice. According to this model, the optimum of the process (i.e., the greatest amount of beet juice) should be obtained for E_0 = 2.99 kV/cm, n_0 = 128 and T_0 = 30 μs (Figure 13).

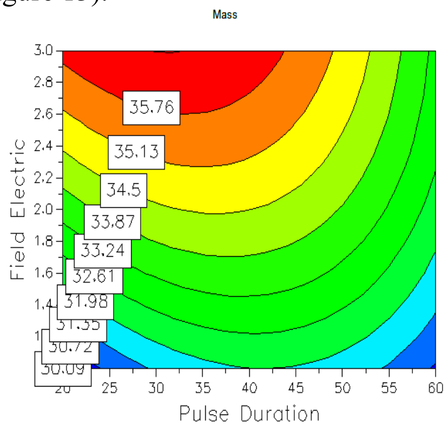


Fig. 12. *Response contour plots of the response for n = 100*

	Field Electric	Pulse Duration	Pulses number	Mass	iter	log(D)
1	3	30,9105	112,513	36,501	103	-10
2	2,9992	30,6102	128,796	36,5478	78	-10
3	2,9999	38,641	122,979	36,2812	150	-1,2385
4	3	37,9709	79,6409	35,9848	123	-0,3846
5	2,9997	30,4427	133,818	36,5465	86	-10
6	2,9992	30,6102	128,796	36,5478	78	-10
7	3	28	150	36,471	0	-10
8	2,9333	34,6667	116,667	36,3725	7	-1,8671

Fig. 13. *Subroutine of MODDE.05 representing the set point*

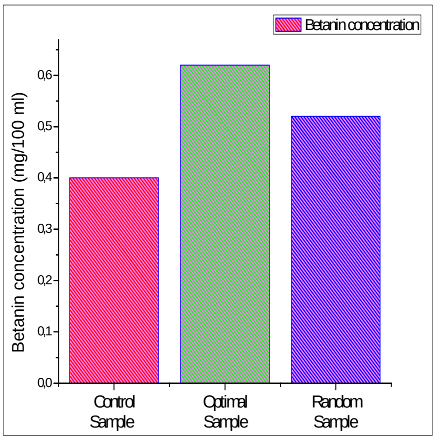
4.1. Analysis of Betanin Concentration

Three samples were analyzed with a spectrophotometer: a control sample, a random point (2 kV/cm, 20 μs and 50 pulses) and a sample treated with the optimal values (E_0 = 2.99 kV/cm, n_0 = 128 and T_0 = 30 μs).

Fresh extracted samples were filtered through two layer cheese cloths, then have been put in 90% methanol (50 ml for each gram). The samples were centrifuged using the Eppendorf 5804R model centrifuge at 3000 rpm for ten minutes. The supernatant was separated and the absorbances were read at λ = 537 and λ = 600 nm on Specord 200 plus spectrophotometer [5].

An obtained result represented in Figure 14 clearly shows a significant increase of betanin concentration for the sample pre-treated with optimal values.

The physico-chemical analysis of different extracts juice have shown that there are significant differences in the concentrations of pigments between the sample treated with PEF and the sample untreated (Figure 14).



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ig. 14. *Concentration of betanin of the three samples*

The results showed that treatment with PEF increases the quality of juice extracted by increasing the concentration of substances in the juice. Moreover, the juice obtained without treatment (control sample) remains less quality than juice treated with PEF.

5. Conclusion

Pulsed Electric Field treatment can be considered an effective non-thermal permeabilization technique in food processing, provided that a careful analysis of process conditions for the specific foodstuff is carried out. Pulsed electric field pre-treatment for the extraction process of beet juice depends on several factors.

According to the experimental analysis performed, it was demonstrated that among the process variables influencing cell permeabilization, the electric field strength and the number of pulses are the most significant. It can be concluded that pulsed electric field treatment had significant influence on the most of investigated parameters.

The results show that the samples that have been CEP free treatment for equal time more substances of betanin than untreated samples.

The results obtained with the treatment of food by pulsed electric fields are encouraging, even if for the moment confined to laboratory scale. Processing speed permitted by this technique is a major advantage.

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