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EXPERIMENTAL MODELLING THE APPLICATION OF PULSED ELECTRIC FIELD FOR ENHANCEMENT OF BETANIN AND JUICE EXTRACTION USING RSM TECHNIQUE

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ABSTRACT

A procedure based on Response Surface Modeling (RSM) is applied for identifying the set point of the juice extraction process using pulsed electric field pre-treatment. The experiments were carried out on a laboratory experimental bench of beet juice extraction, in which three factors of the process were analyzed: the electric field level E (kV/cm), the number of pulses n and the pulse duration T (μs). Three "one-factor-at-a-time experiments", followed by a composite design, were performed following a well defined experimental procedure. A set point was identified corresponding to the optimal values of the above-cited factors.

1. Introduction

PEF treatment can prove to be useful for the pasteurization of liquid foods (Maciej et al. 2005; Grahl and Markl 1996; Heinz et al.2002; Hulshegeret al.1981; Nabil et al. 2011) and the recovery of desired substances from plant cells without the use of chemical or thermal treatment (Schilling et al. 2008; Ade-Omowaye et al. 2001; Robert et al. 2009). Plant cell cultures producing secondary metabolites are permeabilized by electroporation by moderate high voltage pulsed electric field (1-5 kV/cm).

Traditional process of fruit juice production consists of mechanical expression by pressing or decanting combined with enzyme pre-treatment of the mash (Jia et al. 1999; Tanya et al. 2006). Such pre-treatment can damage cell walls of tissues. To obtain higher juice yield and to reduce the processing time, the manufacturers apply higher treatment temperatures at the mash stage. However, enzyme and thermal treatments are always accompanied by high energy consumption and degradation of juice quality, loss of the vitamins and changes in colour and flavor (Massimiliano et al. 2013).

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, 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 setup, 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. Materials and methods

2.1. Pulsed electric field (PEF) for electroporation

Electroporation refers to the ability of electric fields to cause the formation of reversible or irreversible pores in the membranes of cells. Exposing a biological cell (plant, animal and microbial) to a high intensity electric field (kV/cm) using very short pulses (μs to ms) induces the formation of temporary or permanent pores on the cell membrane (Figure 1). This phenomenon 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.

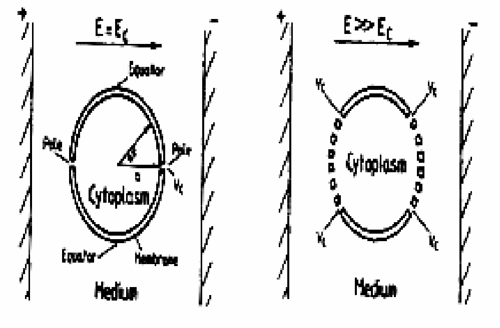


Figure 1. Schematic diagram of a cell exposed to electric field

2.2. Treatment chamber

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 of an insulated cylinder made of plastic (Teflon, PTFE) of length 140 mm and diameter 70 mm (Figure 2). The electrodes are constituted by 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. For all experiments, the same treatment chamb was used for both pressing and pulsed electric field treatment steps.

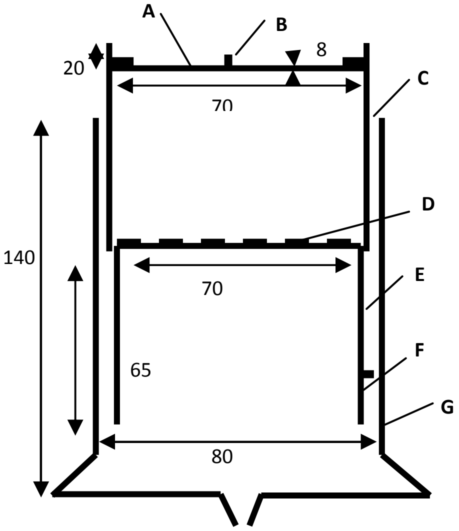


Figure 2. Schematic description of the treatment chamber (all dimensions are in mm).
A- Stainless Steel disk (upper electrode),
B – High voltage connection, C - Teflon cylinder, D – Stainless steel sieve,
E - Perforated stainless steel plunger (lower electrode), F - Ground connection, G- Plastic container for the collection of extracted juice

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.

2.3. Pulse generator

Pulsed electrical field treatment was achieved by using a PEF generator, represented in Fig. 3. The input voltage was regulated by the autotransformer. The voltage was delivered by a direct current high voltage supply (DC-HV) (35 kV, 30 mA), comprising a step-up transformer and a bridge of rectifying diodes. A variable autotransformer (AT) (Langlois ALT5A) was used to supply the desired voltage to the treatment chamber. A 10-kΩ resistor (R) was used to limit the current passing through the bank of capacitors used to store the energy. A spark-gap switch was used to discharge the energy stored in the condenser into the treatment chamber. Figure 4 represents the experimental setup used for this work.

2.4. 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.

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**.**

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, 30 µs, 100 kg/ cm^2	40.6 g
Untreated sample	100 kg/ cm^2	21.3 g

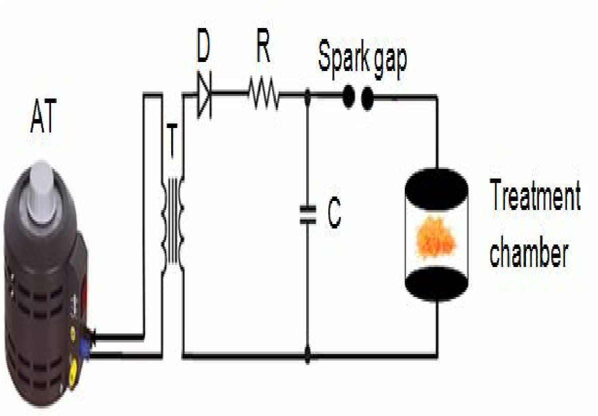


Figure 3. Electrical circuit of the pulse generator

Figure.4. The experimental setup
1. Auto-transformer- 2. DC-HV power supply- 3. Electrostatic voltmeter- 4. HV measuring probe 5. Charging resistors- 6. HV charging capacitor- 7. HV discharge switch-
8. Treatment chamber

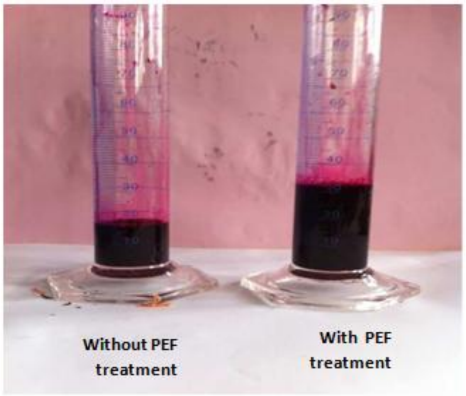


Figure 5. PEF treated and Untreated juice extraction

2.5. Experimental Designs Methodology

The Composite Centred Faces design (CCF), which gives quadratic models, was adopted. The quadratic dependence between the output function to optimize (response) and the input variables u_i (i = 1,......................,k) (factors) has the following expression (Eriksson et al. 2000; Frigon and Mathews, 1996; Taguchi, 1987):

$$y = f(u_i)c_0 + \sum c_i u_i + \sum c_{ijCij} + \sum c_{ii} u_i^2 \qquad (1)$$

As Δu_i and u_{i0} are respectively the step of variation and the central value of factor *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, we obtain:

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

A Windows program dedicated to experimental designs was used (MODDE 5.0 software, Umetrics AB, Umea, Sweden) (MODDE 5.0).

2.6. Design of PEF juice extraction experiments

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 half of the amplitude (Fig.6).Thus, following values were obtained: T = 30 µs for C = 1 µF, T = 55 µs for C = 2 µF and T = 80 µs for C = 3 µF.

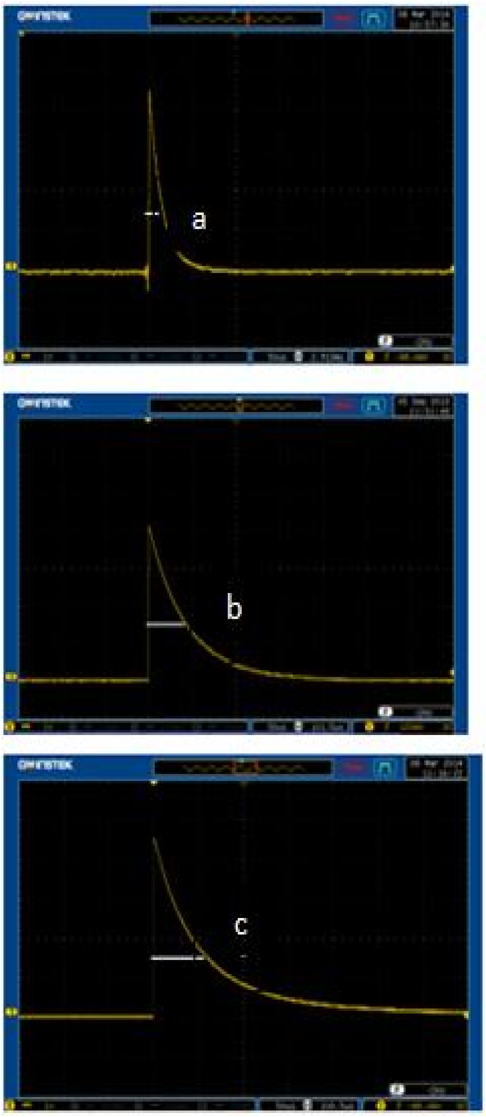


Figure 6. Current waveforms delivered by the pulse generator for different values of capacitance C
a. T= 30 µs (C = 1 µF), b. T= 55µs (C = 2 µF), c. T= 80 µs (C = 3 µF)

3. Results and discussions

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 = 30 µs.

Experiment 2: Variable number of pulses n (50-200), at constant values of E = 2,5 kV/cm and T = 30 µs.

Experiment 3: Variable pulse duration T (30-80 µs) 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. Obtained results of Experiments 1–3 are represented in Figures 9 - 11. The mass of extracted juice and the absorbance were considered as significant for the evaluation of the process and represented as functions of the three control factors. Obtained results in this section served to define the domain of variation of E, n and T to indentify a mathematical model using MODDE 5.0 software.

First, the graph in Figure 9 shows that in the conditions of Experiment 1, the mass of extracted juice and the absorbance increase with increasing pulsed electric field intensity up to 3 kV/cm. Then they decrease for higher values of E. Thus, E_{min} = 2.5 kV/cm and E_{max}= 3.5 kV/cm were retained as the limit values for the electric field (PEF).

In the conditions of Experiment 2 (Figure 10), we noticed the same variation concerning the influence of of pulses number. The mass of extracted juice and the absorbance firstly increased with the pulses number up to n =100 pulses, then they decreased. Consequently, the domain of variation of this factor was defined as n_{min} = 50 and n_{max} = 150 pulses.

Furthermore, results of experiments 3 (Figure 11) obtained according to the pulse duration τ show that τ should not exceed 55 µs. Otherwise the mass of the juice will decrease, causing the diminution of the extraction efficiency. Indeed, when the pulse duration is higher the carrot 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.

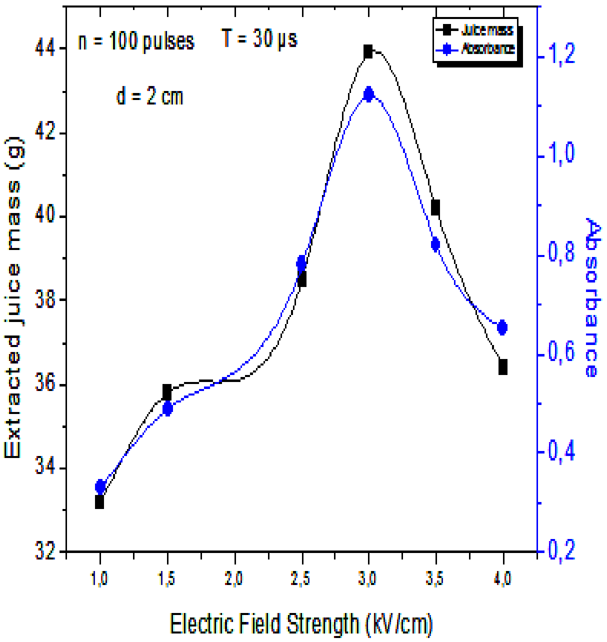


Figure 9. Evolution of extracted carrot juice mass and absorbance according to the voltage (n=100 pulses, T = 30 µs)

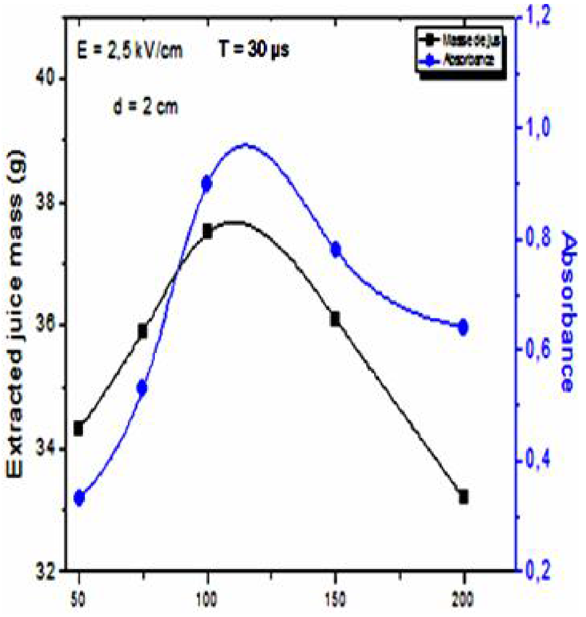


Figure 10. Evolution of extracted carrot juice mass and the absorbance according to the number of pulses (E = 2,5 kV/cm, T =30 µs)

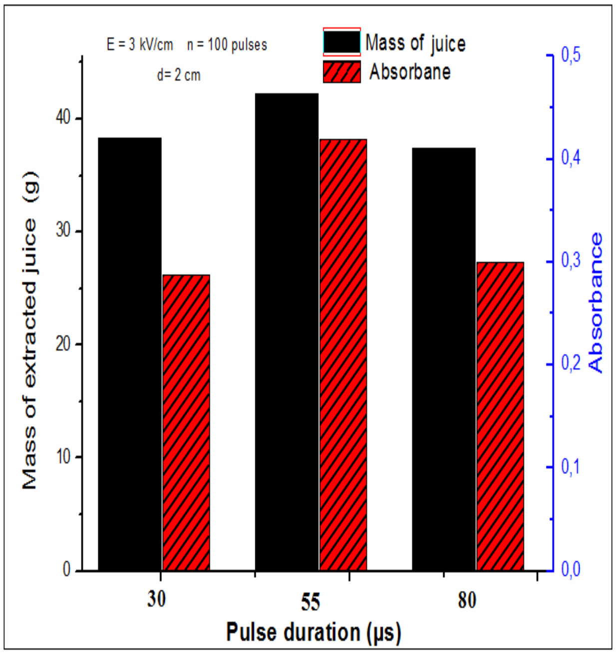


Figure 11. Evolution of extracted carrot juice mass and the absorbance according to the pulse duration
(E = 3 kV /cm, n = 100)

A central CCF design was carried out for identifying the set point (E_0, n_0 and T_0); the two levels "max" and "min" are the limits established in previous section for each of the three input 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:

$$E_c = (E_{\min} + E_{\max})/2 = (2.5 + 3.5)/2 = 3kV/cm \tag{4}$$

$$n_c = (n_{\min} + n_{\max})/2 = (50 + 150)/2 = 100 Pulses \tag{5}$$

$$T_c = (T_{\min} + T_{\max})/2 = (30 + 80)/2 = 55 \mu s \tag{6}$$

The results of all the experiments are given in Table 2. According to all of the experiments modeling software MODDE 5.0 gave us a mathematical model of juice extraction and the absorbance 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 12). MODDE 5.0 also gives the effect of each parameter on extracted juice yield (Figure 13).

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

Exp.N°	E [kV/cm]	T [µs]	n	M [g]	Abs
1	2.5	30	50	39.61	1,031
2	3.5	30	50	36.08	1,287
3	2.5	80	50	40.31	1,215
4	3.5	80	50	34.28	0,71
5	2.5	30	150	40.31	0,488
6	3.5	30	150	38.2	0,577
7	2.5	80	150	39.41	1,21
8	3.5	80	150	35.78	0,52
9	2.5	55	100	41.89	0,996
10	3.5	55	100	36.72	0,714
11	3	30	100	40.82	0,639
12	3	80	100	39.86	0,621
13	3	55	50	40.04	1,349
14	3	55	150	40.28	0,765
15	3	55	100	40.88	0,703
16	3	55	100	40.88	0,703
17	3	55	100	40.88	0,703

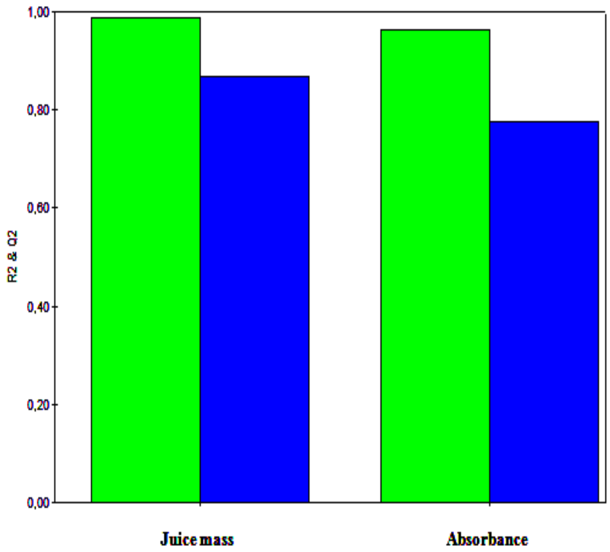


Figure 12. Representation of descriptive quality and predictive quality of mathematical model of juice extraction and the absorbance

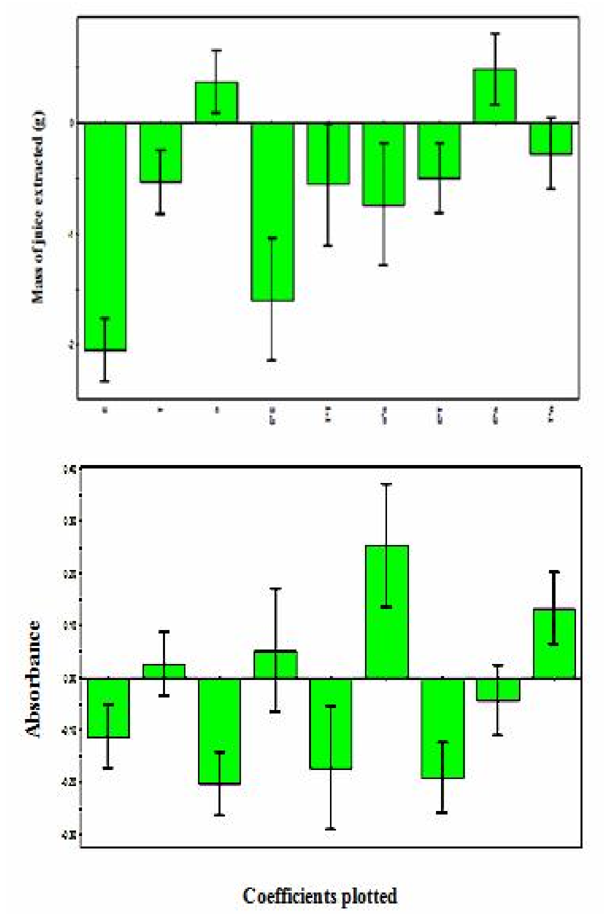


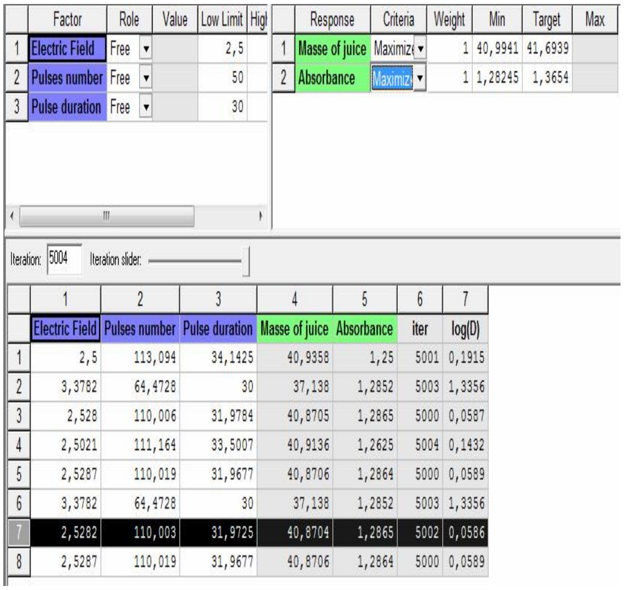
Figure 13. Plotted coefficients of the obtained model

The mathematical model of masse of extracted juice using pulsed electric field (PEF) proposed by modelisation software MODDE 5.0 is presented in equation bellow:

$$M = 40.89 - 2.05E + 0.37n - 0.54T - 1.59E^2$$
$$- 0.74n^2 - 0.56T^2 - 0.50E*T - 0.47E*n \quad (7)$$

$$Abs = 0.760 - 0.11E + 0.025n - 0.20T$$
$$- 0.174n^2 - 0.256T^2 + 0.132n*T - 0.19E*n \quad (8)$$

According to this model, the optimum of the process (i.e. the greatest amount of carrot juice) (Figure 14) should be obtained for Electric field E_0=2.5 kV/cm, number of pulses n_0= 110 and pulse duration T_0 = 31 µs.



	Factor	Role	Value	Low Limit	Higl
1	Electric Field	Free		2,5	
2	Pulses number	Free		50	
3	Pulse duration	Free		30	

	Response	Criteria	Weight	Min	Target	Max
1	Masse of juice	Maximiz	1	40,9941	41,6939	
2	Absorbance	Maximiz	1	1,28245	1,3654	

Iteration: 5004 Iteration slider:

	1	2	3	4	5	6	7
	Electric Field	Pulses number	Pulse duration	Masse of juice	Absorbance	iter	log(D)
1	2,5	113,094	34,1425	40,9358	1,25	5001	0,1915
2	3,3782	64,4728	30	37,138	1,2852	5003	1,3356
3	2,528	110,006	31,9784	40,8705	1,2865	5000	0,0587
4	2,5021	111,164	33,5007	40,9136	1,2625	5004	0,1432
5	2,5287	110,019	31,9677	40,8706	1,2864	5000	0,0589
6	3,3782	64,4728	30	37,138	1,2852	5003	1,3356
7	2,5282	110,003	31,9725	40,8704	1,2865	5002	0,0586
8	2,5287	110,019	31,9677	40,8706	1,2864	5000	0,0589

Figure 14. Subroutine of MODDE.05 representing the set point

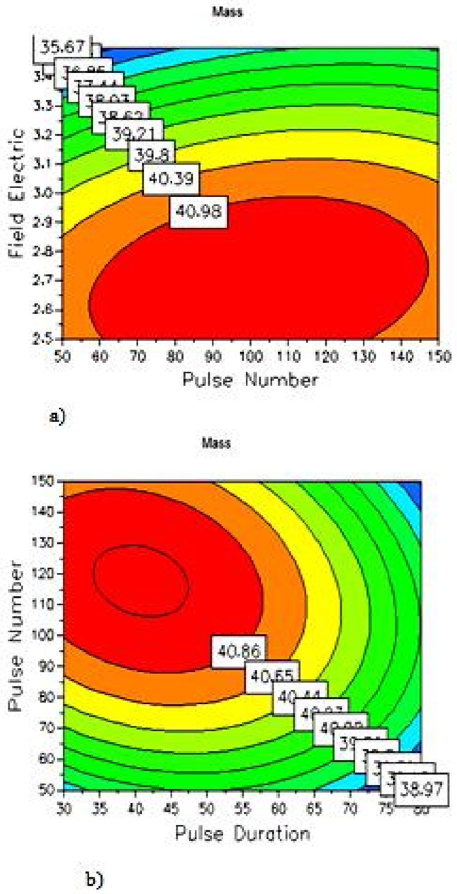


Figure 15. Response contour plots for middling.

Figure 15 shows the iso-response contours obtained with the present model; figure 15. represents the dependency of the middling mass of PEF extracted juice according to the variation of both treatment unit factors (i.e., the electric field level, the pulses number and pulse duration or pulse with).

3.1. Analysis of betanin concentration

Three samples were analyzed with a spectrophotometer: a control sample, a random point (1.5 kV/cm, 30 μs and 50 pulses) and a sample treated with the optimal values (E_0 = 2.5 kV/cm, n_0 = 110 and T_0 = 31 μs).

Fresh extracted samples were filtered through two layer cheese cloths andthen 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 absorbance was measured at λ= 537 nm and λ= 600 nm on Specord 200 plus spectrophotometer (Sükran et al., 1998). An obtained result represented in Figure 16 clearly shows a significant increase of betanin concentration for the sample pre-treated with optimal values.

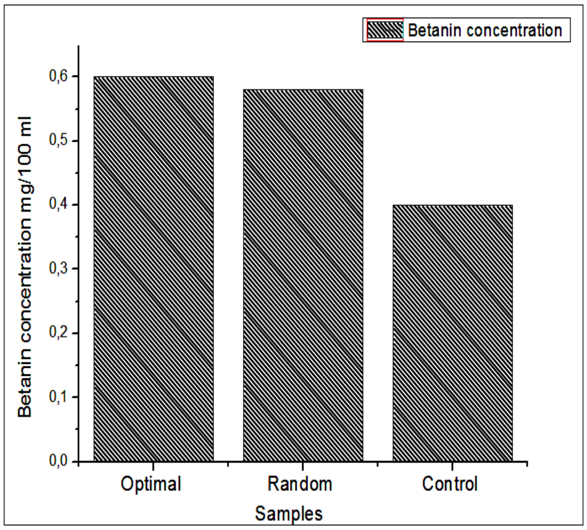


Figure 16. Concentration of betanin of the three samples

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 12). 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.

4. Conclusions

Today's juice extraction industry needs to increase the quantity of production while maintaining the same quality of the juice. Application of PEF would assist the industry. From the conducted experiments and analysis we can conclude that PEF treatment resulted in the breakdown of the cell membrane which in turn enhanced juice extraction more than the untreated sample. PEF treatment increased the quantity of substances contained in juice extracted which increased the quality of juice extracted using a pulsed electric field.

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