EXPERIMENTAL INVESTIGATION A SIMULTANEOUS APPLICATION OF PULSED ELECTRIC FIELD AND PRESSURE FOR JUICE EXTRACTION

Yassine BELLEBNA Hamza BERMAKI Abedelkader SEMMAK Amar TILMATINE

Department of electrical engineering
University of Djillali Liabes, Sidi Bel Abbes, ALGERIA
Email: yassinebellebna@yahoo.fr

Abstract: *Pulsed electric field (PEF) of microsecond duration and high intensity (in the kilovolt-per-centimeter range) has the ability to trigger functional modifications in biological cells. Although the biophysical mechanisms underlying the induced biological effects are not yet clear, promising applications have been found in biology, medicine and environment. 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 on juice extraction from a vegetable food was investigated in this work. This paper is aimed to analyze the efficiency of the PEF pretreatment of carrot juice extraction process even when the control factors undergo slight random variation. The experiments were carried out on a laboratory experimental bench. The work concerned the choice of three factors which are the high voltage level, the number of pulses and the pulse duration. Obtained results pointed out that investigated parameters of PEF treatment have significant effect not only in juice yield, but also for improvement of the chemical quality of final product.*

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

1. Introduction

Food industry is continually increasing because of worldwide demand [1-2]. Mechanical expression (hydraulic pressing) is widely used in the processes of solid-liquid separation for extraction of fruit juices and vegetable oils, dewatering of fibrous materials, etc. On the other hand, cell membrane acts as a physical barrier in removing the intracellular substances (water, juices and solutes) from plant food tissues in solid-liquid extraction and drying. Thus, the permeabilization of the cell membrane in a plant food tissue causes the release of intracellular water and solutes (secondary metabolites) to migrate in an external medium [3].

Presently, the rupture of the cell membrane can be obtained by means of several methods according to the desired degree of disintegration and to the particular application. It is possible to identify: thermal and non-thermal methods. High temperature is used in food preservation and in pre-treatment and complementary stages before extraction processes. In this way it is possible to achieve a high degree of cell membrane breakdown, but due to the thermal denaturation of the cell membrane induced by heating; this treatment damages sensory properties [4-5].

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. It is considered as an unconventional method for iquid and food products extraction, which is efficient for juice yield intensification and for improving the product quality in juice production [6-7], processing of vegetables and plant raw materials [8-9], food stuffs processing [10], winemaking [11] and sugar production [12-13].
In the last few years, several studies have demonstrated the ability of intense treatments to obtain safe and shelf-stable liquid foods. Further, novel applications such as improvement of mass transfer processes or generation of bioactive compounds by using moderate field strengths are under current development [14-15].

One of emerging and promising method is the combined PEF and pressure application, which demonstrates significant yield intensification for juice extracted from apples and beets and clarification of the extracted juice. But the major problem arising from simultaneous application of mechanical expression and PEF treatment is the choice of optimal modes of treatment.
The difficulty of the problem is that PEF-treated juice extraction process depends on multiple factors. The list of factors influencing the outcome of the process includes the inter-electrodes interval, the high voltage level, the electric field, the pulse duration, the number of pulses, the average power, the pressure level and so on. The aim of the present study is the application of an

experimental procedure based on RSM (Response Surface Modeling) for modeling this process, using a laboratory experimental set-up. This method was successfully used in other research fields [16-17].

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). 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.

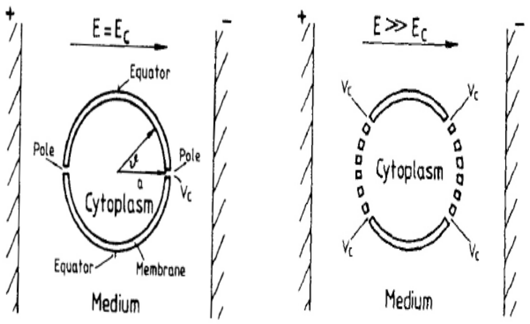


Fig 1. Schematic diagram of a cell exposed to electric field

3. PEF processing system

A PEF system for food processing in general consists of three basic components: a high voltage pulse generator, a treatment chamber and a control system for monitoring the process parameters (figure 2).

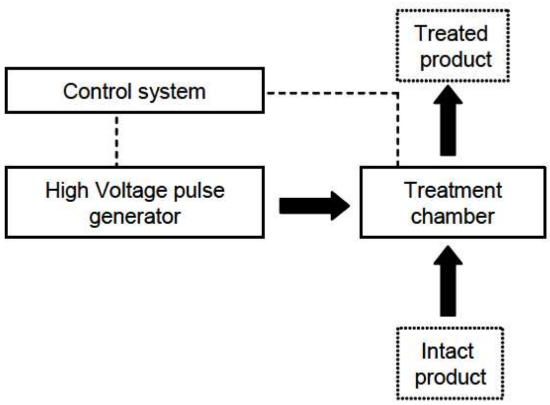


Fig 2.Descriptive representation of a pulsed electric field system for food processing.

4. Materials and Methods

4.1. The high voltage pulse generator

The 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 (figure 3). 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 (figure 3).

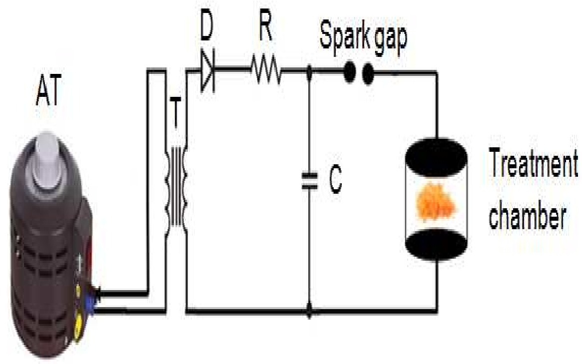


Fig 3. Electrical circuit of the pulse generator

4.2. The treatment chamber

Figure 4 represents a schematic diagram of the treatment chamber. The treatment chamber consisted of an insulated cylinder made of plastic (Teflon, PTFE), 140 mm in length and 70 mm in diameter. The electrodes are constituted by a cylindrical plunger and a disc base of 70 mm diameter having a rigid structure for the 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 ml.

Approximately 100-mm long fresh carrot stems, each of average mass 60 g, were obtained at the 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. Before each experiment, the mash was properly mixed to obtain a homogenous mixture. It was found that the initial moisture content in the mash was of 82% wet basis

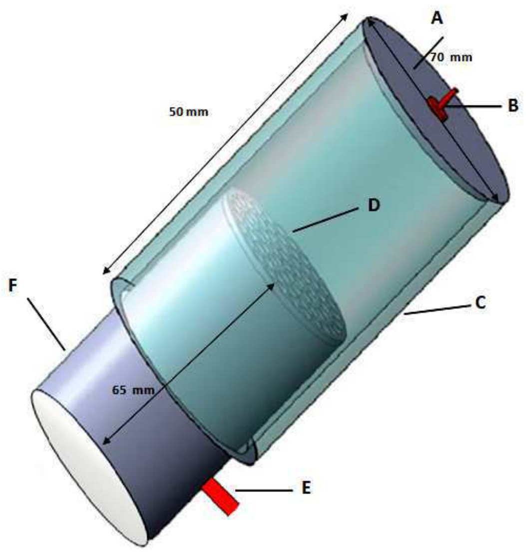


Fig.4. Schematic description of the treatment chamber (All dimensions are in mm).
(A) Stainless Steel disk(upper electrode), (B) High voltage connection, (C) Teflon cylinder, (D) Stainless steel sieve, (E) Ground connection, (F) Perforated stainless steel plunger (lower electrode).

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 mg precision was used to weigh the carrot juice collected in flacon tubes. All the experiments were carried out in stable climatic conditions of temperature (20-25 °C) and humidity (40-60 %).

4.3. The experimental set-up

For all experiments, the same treatment chamber was used for both pressing and pulsed electric field treatment tests. Pulsed electrical field treatment of the carrot mash was achieved by using a PEF generator, represented in Figure 5. 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.

Fig 5. The experimental set-up.

(1) 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

5. Results and discussion

PEF pre-treatment of carrot tissue is followed by the application of a 100 kg/cm^2 pressure, 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 6. Obtained results represented in table 1 show that pulsed electric field treatment increases the quantity of extracted carrot 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 carrot juice extraction

Samples of carrot	Treatment parameters	Mass of extracted juice
PEF-treated sample	3 kV/cm, 100 pulses, 30 µs	40.6 g
Untreated sample	100 Kg /cm^2	21.3 g

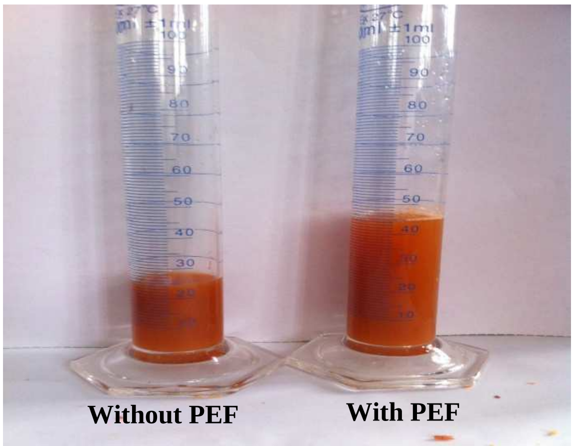


Fig 6.PEF treated and Untreated juice extraction

6. Experimental Designs Methodology

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 [18-20].

Before starting the experiments, it is necessary to set the best and suitable design which can model 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 $(i = 1,.....,k)$ (factors):

$$Y= f(u_i)\ C_0 + \sum C_i u_{in} + \sum C_{ij} u_i u_j + \sum C_{ii} u_i^2 \qquad (1)$$

Knowing that 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, the output function becomes:

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

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.

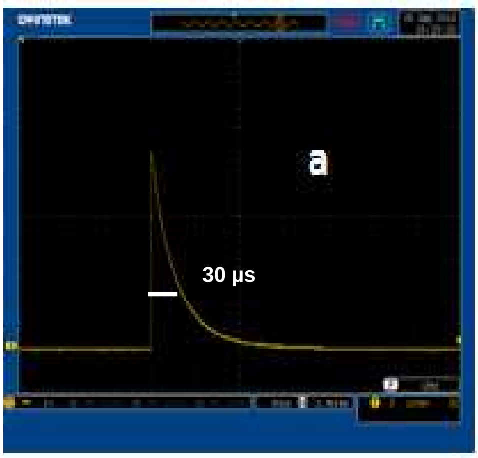
MODDE 5.0 software (Umetrics AB, Umea, Sweden) was used, which is a Windows program for the creation and the evaluation of experimental designs [21]. The program assists the user for interpretation of the results and prediction of the responses. It calculates the coefficients of the mathematical model 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.

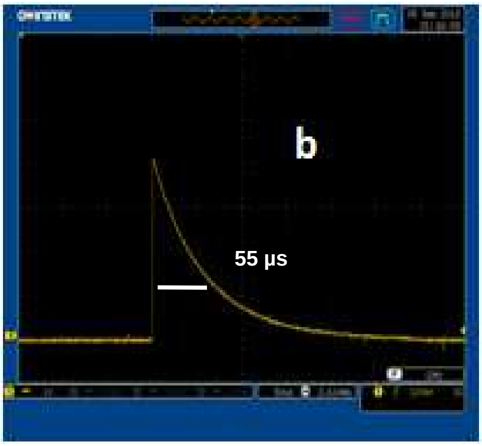
6.1. Experimental design of PEF-treated juice extraction process

Design of methodology for experiments is useful for screening, optimization and robustness testing. Screening experiments are designed to identify the variation of the mass of extracted carrot juice accordingto the following factors:

1. Pulsed 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. Thus, the 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. Given that pulses are of exponential decay shape, the pulse duration is calculated at 37% of the amplitude of the electric field (Figure 7).





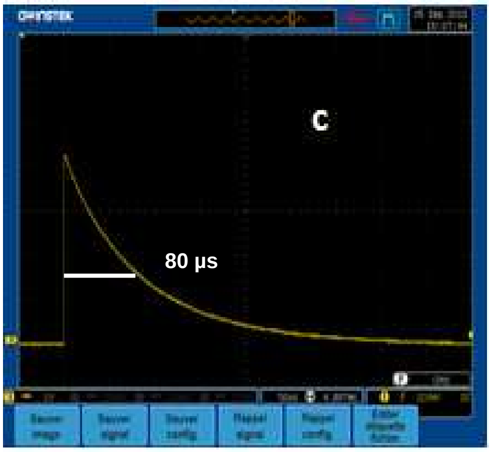


Fig 7.Current waveforms delivered by the pulse generator for different values of capacitance C

(a) T = 30 µs (C = 1 µF), **(b)** T = 55 µs (C = 2 µF), **(b)** **(c)** T = 80 µs (C = 3 µF

7. Experimental 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 = 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 (30-80µs) of the pulse generator at constant values of E = 3 kV/cm and n = 100 pulses.

Obtained results in this section served to the definition of the variation domain of E, n and T.
Obtained results of Experiments 1–3 are represented in Figures 8 - 10. The mass of extracted juice was 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.

The graph in Figure 8 shows that in the conditions of Experiment 1, the mass of extracted juice increases with increasing pulsed electric field intensity up to 3 kV/cm, and then it decreases 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).

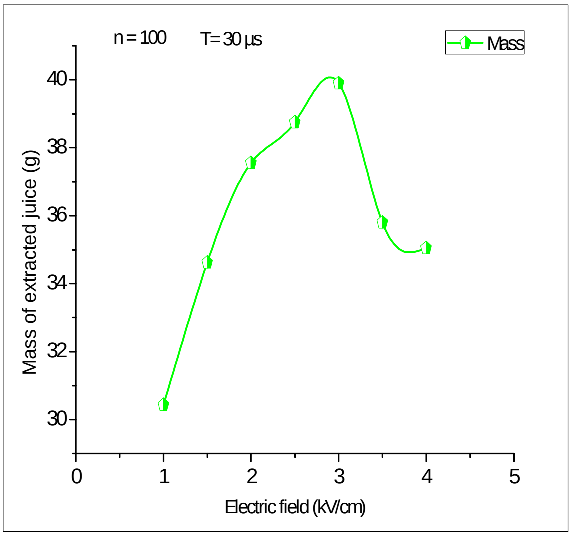


Fig 8.Evolution of extracted carrot juice mass according to the voltage (n=100 pulses, T = 30 µs)

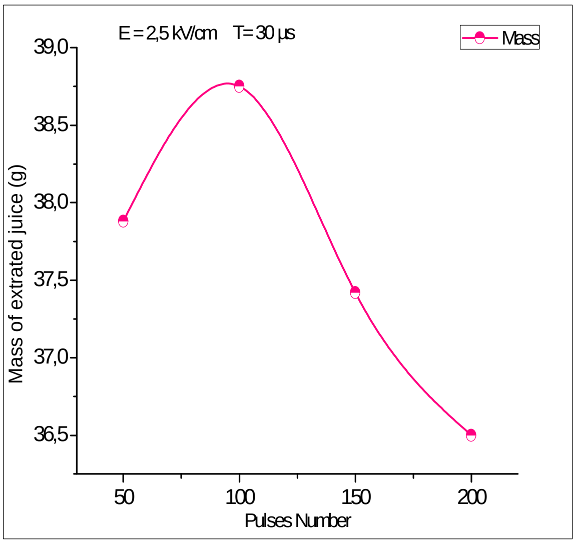


Fig 9. Evolution of extracted carrot juice mass according to the number of pulses (E = 2,5 kV/cm, T =30 µs)

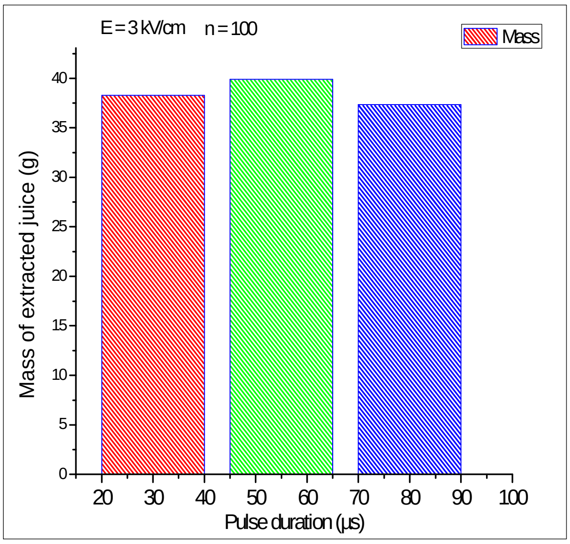


Fig 10.Extracted carrot juice mass for three values of the pulse duration (E = 3 kV /cm, n = 100)

In the conditions of Experiment 2 (Figure 9), we noticed the same variation concerning the influence of pulses number *n*. The mass of extracted juice firstly increased with *n* 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.

Furthermore, results of experiments 3 (Figure 10) obtained according to the pulse duration T show that T 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 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:

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

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

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

The results of all experiments are given in Table 2.

Table 2.Obtained results of the CCF experimental design

Exp. N°	E [kV/cm]	T [µs]	n	Masse of juice [g]
1	2.5	30	50	39.61
2	3.5	30	50	36.08
3	2.5	80	50	40.31
4	3.5	80	50	34.28
5	2.5	30	150	40.31
6	3.5	30	150	38.2
7	2.5	80	150	39.41
8	3.5	80	150	35.78
9	2.5	55	100	41.89
10	3.5	55	100	36.72
11	3	30	100	40.82
12	3	80	100	39.86
13	3	55	50	40.04
14	3	55	150	40.28
15	3	55	100	40.88
16	3	55	100	40.88
17	3	55	100	40.88

The mathematical model of extracted juice mass pre-treated by pulsed electric field (PEF) proposed by software MODDE 5.0 is presented in bellow equation and in figure 13 as plotted by MODDE.05. MODDE 5.0 also gives the effect of each factor on extracted juice yield (figure 11).

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

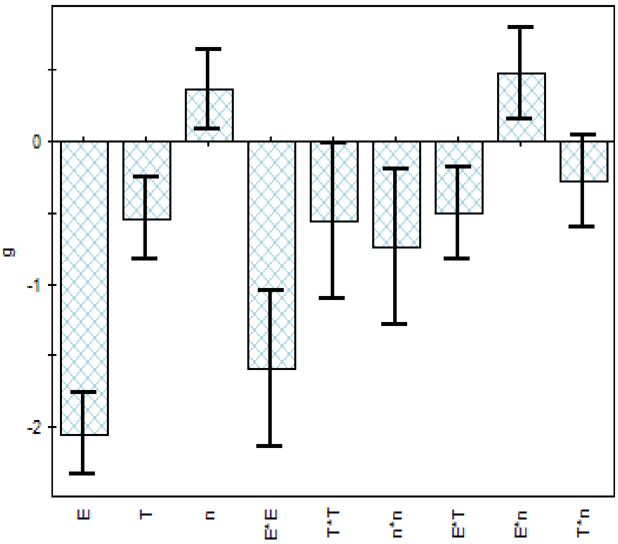


Fig 11. Plotted coefficients of the obtained model

As can be seen in Fig.11, the electric field effect is more significant than the two other factors.

Furthermore, increasing the electric field and the pulse duration will cause the decrease of the extraction yield. Indeed, if a greater amount of energy, which is function of the three factors, is delivered to the cells, a reverse effect will be produced causing the decay of the PEF treated extraction process.

According to this model, the optimum of the process (i.e. the greatest amount of carrot juice) should be obtained for Electric field E_0 =2.7 kV/cm, number of pulses n_0 = 105 and pulse duration T_0 = 49 µs (figure 13). Figure 12 represents the iso-response contours obtained with the present model; figure 12.a represents the dependency of the PEF extracted juice mass according to the variation of both the electric field level and the pulses number, while figure 12.b concerns the variation of the mass according to the electric field level and pulse duration. According to these figures, as deduced from the model, we notice that the electric field is the most significant factor in comparison with pulses number and pulse duration.

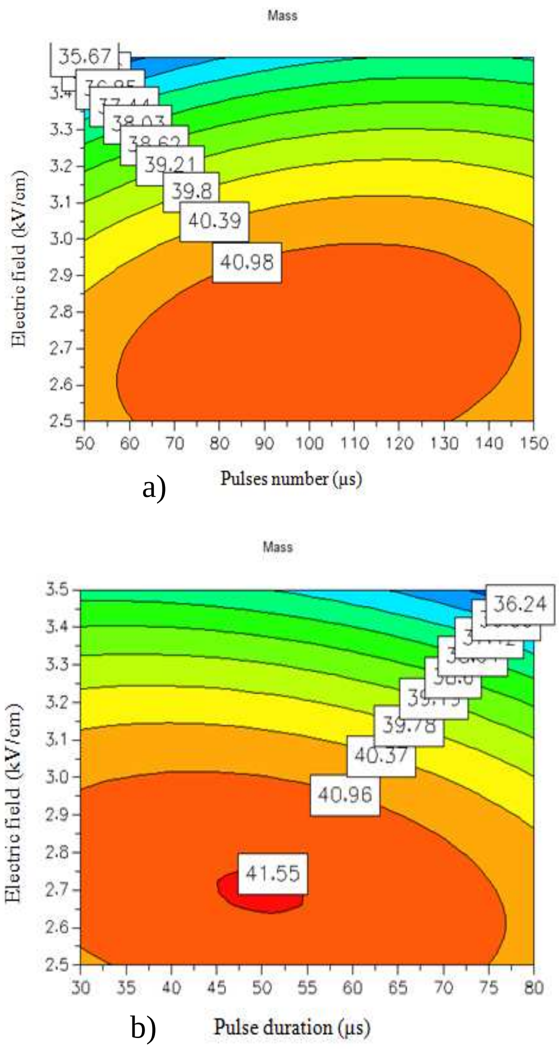


Fig. 12. Response contour plots for extracted juice mass

Iteration: 5003 Iteration slider:

	1	2	3	4	5	6
	Field Electric	Pulse Duration	Pulse Number	Mass	iter	log(D)
1	2,7036	48,6004	105,179	41,577	2946	-1,5542
2	3,4	30,0002	60	37,3212	5000	1,5915
3	2,7094	49,0718	105,203	41,577	755	-1,5546
4	3,4	75	150	36,9108	5001	1,6695
5	3,1	60	76,9422	39,9232	5003	0,8063
6	3,4	30,0002	60	37,3212	5000	1,5915
7	2,7025	49,0567	104,443	41,577	518	-1,5543
8	3,4	75	150	36,9108	5001	1,6695

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

Moreover, in addition to the estimation of the extraction efficiency, a physic-chemical analysis was performed in order to check whether really the mass of the pigments is increased.

Two samples of PEF treated and control carrot mash were filtered through two layers of fabric of cheese, and then were mixed with 90% methanol (50 ml for each gram). The samples were centrifuged using centrifuge (model Eppendorf 5804R 3000 rpm) for ten minutes. The supernatant was separated and the absorbance was measured in the visible spectrum at 400-700 nm using a spectrophotometer (SPECORD 200 plus).The amount of these pigments was calculated according to the method of the Spectrophotometric [22].

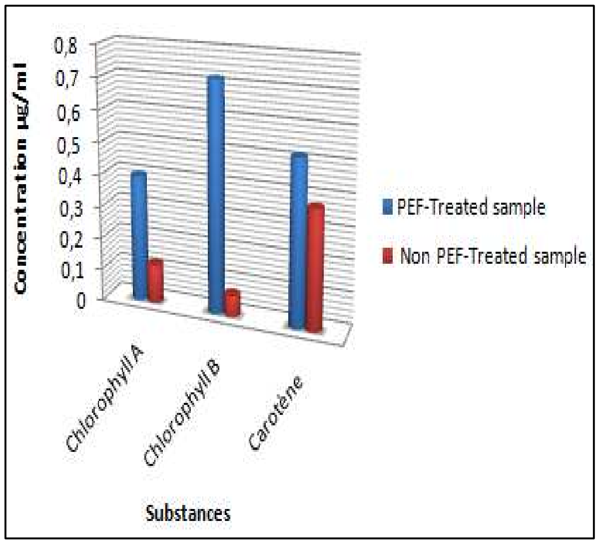


Fig14.Qualitative comparison between the PEF treated sample and non treated sample

According to the obtained results plotted in

Fig.14, there is a significant difference in terms of pigment concentrations between the PEF treated and the control samples. PEF treatment increases the amount of extracted juice by increasing the concentration of substances.

8. Conclusion

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. The methodology of experimental designs is useful to deliver a mathematical model for this process

References

1. Kenneth e. Barlow "*Food and Health*". International Journal of Environmental Studies, volume 17, issue 1,1981. Pages 13-17**.**

2. Jolocam Mbabazi "Environment *and Food"*, International journal of environmental studies, volume 69, issue 6, 2012. Pages 997-998.

3. Akinlaja J., & Frederick, S. (1998). "*The Breakdown of Cell Membranes by Electrical and Mechanical Stress"*, Biophysical Journal, 75(1), 247-254.

4. Zhang, Q., Chang, F. J., Barbosa-Cánovas, G. V. and Swanson, B. G. ."*Inactivation of microorganisms in semisolid model food using high voltage pulsed electric fields"* Lebemsm. Wiss.u.-Technol. 1994a, 27: 538-543.

5. Barbosa-Cánovas, G. V., Swanson, B. G., Chang F. J. and Qin, B. L. " *Non-thermal inactivation of Saccharomyces Cerevisiae in apple juice using pulsed electric fields"* Lebemsm.- Wiss.u.-Techno.l 1995, 28: 564-568.

6. Grishko, A.A., Kozin, V.M., &Chebanu, V.G.." *Electroplasmolyzer for processing plant raw material"* , US Patent no. 4723483, 1991.

7. Gulyi, I.S., Lebovka, N.I., Mank, V.V., Kupchik, M.P., Bazhal, M.I., Matvienko, A.B., &Papchenko, A.Y.." *Scientific and practical principles of electrical treatment of food products and materials* " Kiev: UkrINTEI (in Russian), 1994.

8. Heinz, V., Angersbach, A., & Knorr, D. " *High electric field pulses and membrane perméabilisation* " In Proceedings of the European Conference on Emerging Food Science and Technology, Tampere, Finland, 22-24 November 1999, 34.

9. Rastogi N.K." *Application of High Intensity Pulsed Electric Fields in Food Processing* ", Food Reviews International 19(3): 229-251, 2003.

10. Jones, G.C." *Cossette pretreatment and pressing* ",International Sugar Journal , 90(1077), 157-167, (1988).

11. Kalmykova, I.S. " *Application of electroplasmolysis for intensification of phenols extracting from the grapes in the technologies of red table wines and natural juice* ". PhD Thesis, Odessa Technological Institute of Food Industry, Odessa, Ukraine (in Russian), 1993.

12. Knorr, D., &Angersbach, A."*Impact of high intensity electric field pulses on plant membrane perméabilisation* ".Trends in Food Science and Technology, 9, 185-19, 1998.

13. Knorr, D., Geulen, M., Grahl, T., &Sitzmann, W.." *Food application of high electric field pulses* ", Trends in Food Science & Technology, 5, 71-75, 1994.

14. Lanoiselle, J.L., Vorobiev, E.I., Bouvier, J.M. &Piar G..*Modeling of Solid/Liquid Expression for Cellular Materials,* AIChE Journal, 42, 2057-2068, 1996.

15. Robert Soliva-Fortuny, Ana Balasa, Dietrich Knorr, and Olga Martın-Belloso." *Effects of pulsed electric fields on bioactive compounds in foods* ", a review. Trends in Food Science & Technology 20 (2009) 544-556.

16. Dascalescu L., Samuila A., Mihalcioiu A., Bente S., and Tilmatine A."*Robust control of electrostatic separation processes* ", IEEE Transactions on Industry Applications. vol. 41, no. 3, pp. 715–720, May/Jun. 2005.

17. A. Tilmatine, S. Bendimerad, M. Younes and L. Dascalescu."*Experimental analysis and optimisation of a free-fall triboelectric separator of granular plastic particles* " . International Journal of Sustainable Engineering, Vol. 2, No. 3, September 2009, 184–191

18. Frigon, N.L. and Mathews, D., *Practical Guide* to Experimental Design, 1st Edn.,Wiley, New York (1996).

19. Taguchi, G., System of Experimental Designs, Kraus International Publications, New York (1987).

20. Eriksson L., Johansson E., Kettaneh-Wold N., Wikström C. and Wold, S. *Design of Experiments. Principles and Applications,* Learnways AB, Stockholm (2000).

21. MODDE 5.0, ''*User guide and tutorial*'', Umetrics (1999).

22. Sükran DERE,Tohit GÜNES, Rıdvan SIVACI. Spectrophotometric "*Determination of Chlorophyll - A, B and Total Carotenoid Contents of Some Algae Species Using Different Solvents*»,Journal of Botany, 13-17,1998