Application of pulsed electric field for oil extraction from sunflower seeds: Lab scale parameters optimization

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Abstract—**Application of pulsed electric field (PEF) has been proposed to increase oil extraction yield from sunflower seeds. PEF treatments have been carried out under various parameters such aselectric field, frequency, pulse width, time of treatment and solvent amount in samples. 5^5 fractional factorial design has been used for optimization of process parameters and impact of the PEF factors on the square model parameters has been investigated. The oil yield was found to increase by 9.1 % after PEF treatment of sunflower seeds for 90 sec under an electric field of 7.0 kVcm^{-1} having frequency of 1.5 Hz and pulse width of 30 µs. The obtained results will be used to scale up non-thermal food processing technologies for oil extraction from sunflower seeds.**

Keywords: sunflower seeds, electroporation, pulsed electric fields, oil extraction, optimization

I. INTRODUCTION

Sunflower (*Helianthus annuus)* oil is the most popular oil produced in Russian Federation, Ukraine, Argentine and some European countries [1]. For example, production of sunflower oil amounted 530 million tons in 2010 and the export exceeded 340 million tons [2]. Sunflower oil is widely used in food processing, cooking, production of soap, mayonnaise, etc.

The percentage of oil in this seeds can range 45% to52% and the protein content is approximately of 20%. The fatty acids content of this oil is composed mainly of linoleics, linolenicsstearics,palmiticmyristicsand arachidic oleics.

Pressing and solvent extraction are the most common processes used for oil extractionfrom seeds. Screw presses are usually used for industrial scale pressing; however the hydraulic presses can also be employed [3]. Gasoline, super critical CO_2, hexane and ethanol are usually used as solvents for extraction [4].

Recently application of green technologies inoil extraction hasyielded inhigh quality products [5]. Pulsed electric field (PEF) treatment is one of the green technologies that has been used as an assisted intensification method for oil extraction. Interest for PEF has beencontinuously increasing. Various studies have investigated the effect of PEF treatment oncell disintegration [6] and extraction efficiency of fruit juices [7].

In reference [8], application of PEF on coconut waterrevealed thatPEF is a promising technology for inactivation of the native micro flora and retention of quality in tender coconut water-nannari extract beverage. Nutritional and sensory attributes losses were minimized.

The application of PEF for oil extraction from oilseeds and its functional ingredients [9] has received increased interest during last ten years. Somereportshave found that PEF pre-treatment could increase oil yield of maize germs [10] and olives [10, 11] by about 32.4% and 6.5-7.4% respectively.

It has been reported that PEF treatment can improve oil yield as well as oil quality of rapeseeds [12]. Further treatments like moisture control of the seeds to increase conductivity of the seed cake during PEF treatment are required.

Furthermore, the synergic effect of thermal and PET treatments on soya PC and DPPC vesicles has been investigated by Sarkis et al. [13]. They found that thermal treatment at certain temperature could enhance efficiency of PEF inactivation.

In summary, many reports have demonstrated application of the PEF for oil extraction from oilseeds as an intensification method, increasing oil yield.Although the effect of PEF assisted intensification on oil yield of various oilseeds has been studied in literature, however PEF-assisted extraction of sunflower seeds, as one of the green technology methods and its impact on oil yield from sunflower seeds is yet to be investigated.

The aim of this work is to find the optimized parameters of PEF treatment, such as electric field, frequency, pulse width, time of treatment and solvent content to apply for scale up of non-thermal food processing technologies for oil extraction from sunflower seeds.

II. MATERIALS AND METHODS

A. Materials

Hulled sunflower seeds grown in 2014 in USA were purchased at a local market (Cold storage, Singapore). Sunflower seeds were cleaned in an air screen cleaner to remove all foreign material. All seeds were stored in dark at

4ºC until further analysis.Bio-ethanol (absolute ethanol, 99.8%, Sigma Aldrich) was used as a solvent to adjust conductivity of samples during PEF treatment.Hexane (n-Hexane, 99%, Tech grade) was used as solvent during oil extraction.

B. *Moisture content*

20 gr of sunflower seeds was weighed on an analytical scale, up to 5 effective digits, in a glass container. Prior to weighing, the glass container was dried in an oven at 103°C for 30 minutes and cooled down in a desiccator to minimize measurement error. The weighed seeds were dried in the oven at 103°C for 48 hours and weighed on the analytical scale. The initial moisture content of sunflower seeds was found to be about 7.95%.

C. *Sample preparation*

Sunflower seeds were groundin an agate mortar, until aparticle size ofless than 0.5 mm.

Conductivity of samples was regulated by a liquid solvent, namely absolute ethanol (99.8%, Sigma Aldrich).

D. *PEF treatment*

Ground sunflower seeds was treated by pulsed electric field (PEF) in a dielectric chamber, havingtwo Titanium top and bottom electrodes (diameter 40 mm) (Fig. 1). Electrode gap was set as 5 mm to reach a treatment volume of about16 cm^3. Square electric pulses were generated by a function generator (Agilent 33220A, USA) and amplified by a high voltage amplifier (Trek COR-A-TROL 610D, USA).

The chamber was filled with ground samples to form a sunflower cake and electrodes were fully covered. Positive trains of pulses were applied to samples. The characteristics of the applied electric pulses such as electric field, frequency and pulse width were monitored using a digital oscilloscope (Tektronix TDS 220, USA) with PHV 621 oscilloscope probe (400 MHz, x100). The experimental setup is shown in Fig 1.

E. *Fractional factorial design*

Different variations of parameters wereused in this study (Fig. 2). As summarized in Table 1, a 5^5 (5 levels and 5 factors) fractional factorial design (FFD) [14] was used. A full replication would take 5^5= 525 runs. Hence, this design is a 1/25th rep = 525/25 = 25 to test several factors with minimum number of trials. The effect of electric field (1, 3, 5, 6, 7 kV cm^{-1}), frequency (0.5 Hz, 1.5 Hz, 5 Hz, 10 Hz, 15 Hz), solvent content in samples (10, 20, 30, 40 and 50wt%),time of treatment (10,30, 60, 90, 120 sec) andpulse width (10,20, 30, 40, 50 µs)on the oil yield was investigated.

As a planning matrix, standard Graeco-Latin square was used [15] as presented in Table 2. The PEFtreatment was done at room temperature, approximately 20°C.

F. *Oil extraction*

Sunflower oil was extracted from the PEF treated samples by solvent extraction using hexane. Oil yield was determined according to the method reported in [16]. The PEF treated sunflower seeds were heated to 40°C for 3 hr in an oven to remove the ethanol. Subsequently, 2.3-2.5gr of samples was placed in a tube and mixed with 40 ml of hexane. The mixtures were shaken in shaker at frequency of 400 min^{-1} for 3hr at room temperature to extract the oil. The extracted oils were filtered using filter paper. Finally hexane was removed by evaporation in a fume hood for 24 hr.

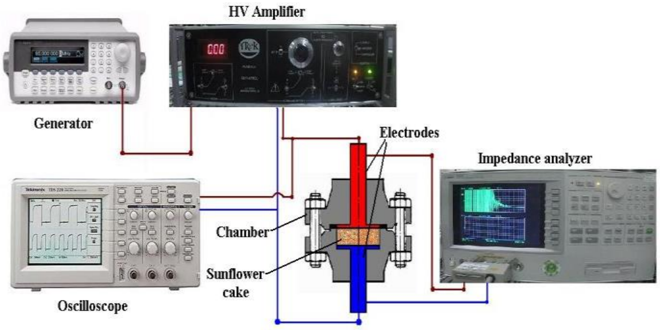

Figure 1.Experimental setup for PEF treatment.

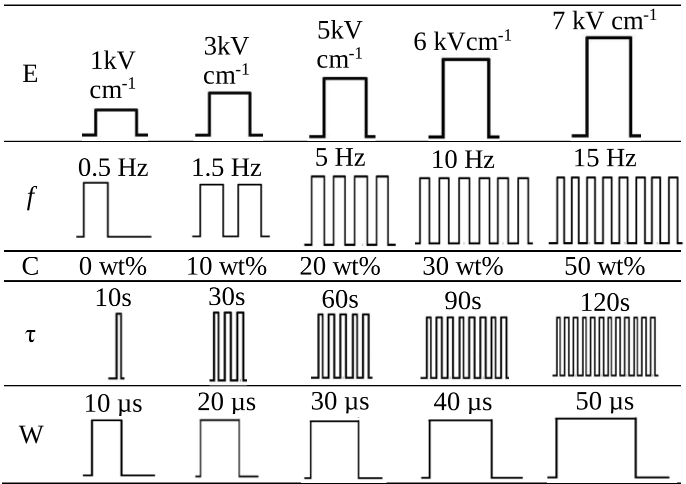

Figure.2Variation of PEF treatment parameters: Electric field (E), frequency (*f*), solvent content (C),treatment time (τ),pulse width (W).

TABLE I. GRAECO-LATIN SQUARE 5^5, a_i- ELECTRIC FIELD, 1 $kVcm^{-1}$(a1), 3 $kVcm^{-1}$ (a2), 5 $kVcm^{-1}$ (a3), 6 $kVcm^{-1}$ (a4), 7 $kVcm^{-1}$ (a5); b_j FREQUENCY, 0.5 Hz (b1), 1.5 Hz (b2), 5 Hz (b3), 10 Hz (b4), 15 Hz (b5);c_q- SOLVENT CONTENT, 10 wt% (c1), 20 wt% (c2), 30 wt% (c3), 40 wt% (c4), 50 wt% (c5); d_k- TIME OF TREATMENT, 10 sec (d1), 30 sec (d2), 60 sec (d3), 90 sec (d4), 120 sec (d5); e_m- PULSE WIDTH, 10 µs(e1), 20 µs(e2), 30 µs(e3), 40 µs(e4), 50 µs(e5).

	b1	b2	b3	b4	b5
a1	c1,d1,e1	c2,d2,e2	c3,d3,e3	c4,d4,e4	c5,d5,e5
a2	c3,d4,e5	c4,d5,e1	c5,d1,e2	c1,d2,e3	c2,d3,e4
a3	c5,d2,e4	c1,d3,e5	c2,d4,e1	c3,d5,e2	c4,d1,e3
a4	c2,d5,e3	c3,d1,e4	c4,d2,e5	c5,d3,e1	c1,d4,e2
a5	c4,d3,e2	c5,d4,e3	c1,d5,e4	c2,d1,e5	c3,d2,e1

G. Impedance and conductance analysis

Impedance and conductance of samples were measured by a precision impedance analyzer (Agilent Technologies, 4294A, USA) using a 4 terminal-pair connection (BNC,16089A Large Kelvin Clip Lead, USA) as shown on Fig. 2. Electrical impedance spectroscopy was conducted at frequencies ranging from 100 Hz to 1 MHz.

Sunflower cake impedance and conductivity were measured and plotted over frequency. Since the phase angle was low, at least at frequencies above 100 Hz, conductivity can be calculated as:

$$\sigma = G{\cdot}l / A \tag{1}$$

where G,l and $A$are conductance, thickness and area of sample respectively.

H. Disintegration index

The cell damage induced by electrical treatment can be indirectly quantified by measuring the electrical conductivity of the sunflower cake. This factorallows determination of the charged particle concentration [17, 18]. The cell disintegration index, Z, can be determined by the following equation:

$$Z = (\sigma - \sigma_i) / (\sigma_d - \sigma_i) \tag{2}$$

In this equation, σ is the electrical conductivity after the treatment, σ_i is the initial electrical conductivity of the samples (which was very close to zero), and$\sigma_d$is electrical conductivity of the maximally disintegrated samples. This equation implies a Z = 0 for an intact tissue and Z = 1 for a completely damaged tissue.

For example, this index has beenreported for different PEF treatment times (1 – 7 ms) for sesame cake [8] and hulled and non-hulled rapeseed [12]. It has been shown that the cell damage increases as a function of PEF treatment time. However, disintegrationsaturated at a certain critical treatment time. The disintegration of PEF treated sesame and hulled and non-hulled rapeseeds reached a maximum of 67 %, 55% and 17% respectively.

I. Statistical analysis

The analysis of the results of the fractional factorial experiments was performed by using the software MS Excel, 2007.The effect of each of the PEF processing factors (electric field, frequency, pulse width, treatment time andsolvent content) on oil yield, as well as their square model parameters were calculated.

All experiments were carried out in triplicate. Average valuesand standard deviations of data were calculated and statistical significance was declared at $p<0.05$ tested by ANOVA.

III. Results and discussion

The sample specificationsshowed that, the seeds used in this work had an oil content of 49.18 ± 0.01% (results expressed in fresh matter basis). The initial moisture content of the material was measured to be about 7.95 ± 0.5%. The oil and protein amounts were similar to those reported by [19].

A. General effect of PEF on oil yield

Fig. 3 represents the effect of PEF processing factors on oil yield of samples. In general, oil yield of sunflower seeds increased after PEF processing. Details of all of the parameters and their value are listed in Table II.

The oil yield was ranging from 39.14% up to 48.24% with its maximum at 23rd experiment, at electric field of 7.0 kVcm^{-1} , frequency 1.5 Hz, pulse width of 30 µs,time of treatment 90 sec and solvent content of 10 wt%.

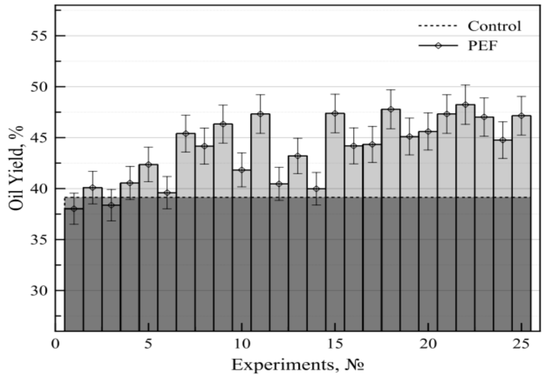

Figure 3. Oil yield for 25 experiments after PEF treatment

TABLE II. Fractional factorial design, 5^5: the effect of different factors on the oil yield, factors: electric field, E, frequency, f,Solvent content, C, time of treatment, τ andpulse width, W.

Exp.	E (kV cm^{-1})	f (Hz)	C (wt%)	τ (sec)	W (µs)	Y (%)
1	1	0.5	10	10	10	38,02
2	1	1.5	20	30	20	40,1
3	1	5	30	60	30	38,37
4	1	10	40	90	40	40,55
5	1	15	50	120	50	42,36
6	3	0.5	30	90	50	39,6
7	3	1.5	40	120	10	45,4
8	3	5	50	10	20	44,17
9	3	10	10	30	30	46,33
10	3	15	20	60	40	41,83
11	5	0.5	50	30	40	47,32
12	5	1.5	10	60	50	40,46
13	5	5	20	90	10	43,21
14	5	10	30	120	20	39,98
15	5	15	40	10	30	47,38
16	6	0.5	20	120	30	44,19
17	6	1.5	30	10	40	44,34
18	6	5	40	30	50	47,78
19	6	10	50	60	10	45,11
20	6	15	10	90	20	45,6
21	7	0.5	40	60	20	47,32
22	7	1.5	50	90	30	48,24
23	7	5	10	120	40	47,03
24	7	10	20	10	50	44,77
25	7	15	30	30	10	47,15

B. *Disintegration index and conductivity of sunflower cake*

PEF treatment increased sunflower cake tissue damage. At electric fields higher than $1 kV.cm^{-1}$ permanent pores were generated and irreversible disintegration happened, which resulted in improved oil yield. From Eq. 2 for intact cells Z=0; for total cell disintegration Z=100%.Fig.4 represents frequency dependence of conductivity of sunflower cakes, with various solvent contents, before and after PEF treatment with electric field strength $5 kV \cdot cm^{-1}$.

Fig. 5illustrates the cell disintegration of PEF treated sunflower seed cakesat different electric fields as well asuntreated samples. With increased electric field, the cell disintegration index of sunflower seeds (Fig. 5), increased to a maximum of 41% at electric field of $7 kV/cm^{-1}$. The better and easier disintegration of sunflower seedcake could be due to the improved electrical conductivity due tothe PEF treatment. Similar disintegration index values were also found for PEF treated rapeseeds[12].

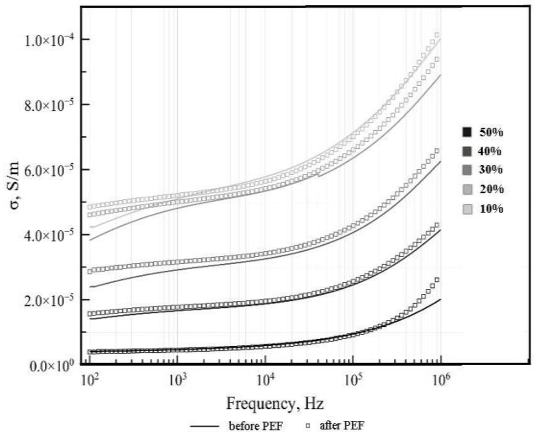

Figure 4.Frequency dependent of conductivity,σ,of sunflower cake recorded for samples with different solvent content.

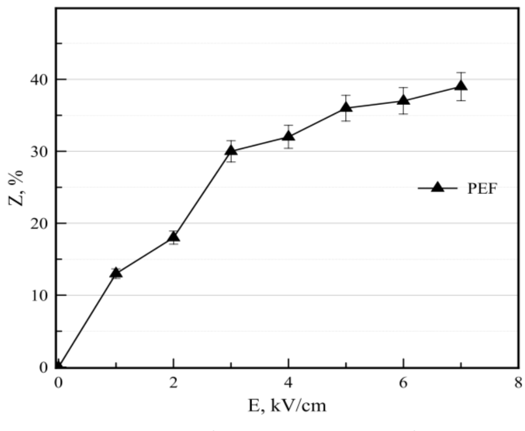

Figure 5.The dependence of conductivity σ on the frequency, recorded at different solvent compounds

C. *Main effects of PEF parameters on oil yield*

Graeco-Latin square 5^5 was analyzed by ANOVA software, using dispersion analysis. Results presented in Table 3. Significance of the impact factors was checked by Fisher criterion, expressed by equation:

$$F = SA_{SOV} / SA_{Error} > F_{table} \quad (3)$$

where SA_{SOV} is square average of source variations (Table 3); SA_{Error} is error square average (Table 3); F_{table} is Fisher criterion table value for degrees of freedom $F_{0.95}(f_1,f_2) = 3.26$, where f_1 and f_2 are degrees of freedom, 4 and 12 subsequently.

TABLE III. DISPERSION ANALYSIS OF GRAEKO-LATIN SQUARE BY ANOVA SOFTWARE

Source of variations (SOV)	Degrees of freedom (DF)	Squares sum (SS)	Squares average (SA)
a	4	5.533	1.383
b	4	0.336	0.084
c	4	2.187	0.546
d	4	1.051	0.262
e	4	0.429	0.107
Error	12	46425.03	5803.1
Total	28	46434.57	5805.5

The Graeco-Latin square design model for oil yield was extracted from the obtained data,by equation [15]:

$$y_{ijqkm} = \mu + \alpha_i + \beta_j + \gamma_q + \delta_k + \zeta_m + \varepsilon_{ijqkm} \quad (4)$$

where μ – denoting the general location parameter of all experiments; α_i , β_j , γ_q , δ_k and ζ_m -denoting the effect for block *i*,*j*,*q*,*k* and *m* respectively; and ε_{ijqkm} - random experimental error.

D. *Estimates for Graeko-Latin square*

To estimate main parameters of square design model, the following equations were used:

$$\begin{aligned} \mu &= \text{average } (Y) \\ \alpha_i &= Y_i - \text{average } (Y) \\ \beta_j &= Y_j - \text{average } (Y) \\ \gamma_q &= Y_q - \text{average } (Y) \\ \delta_k &= Y_k - \text{average } (Y) \\ \zeta_m &= Y_m - \text{average } (Y) \end{aligned} \quad (5)$$

where (from Table 1): average (Y) is the average of all Y for all experiments; Y_i is the average of all Y for which $a = i$, $i=1,..,5$; Y_j is the average of all Y for which $b = j$, $j=1,..,5$; Y_q is the average of all Y for which $c = q$, , $q=1,..,5$; Y_k is the average of all Y for which $d = k$, , $k=1,..,5$; Y_m is the average of all Y for which $e = m$, , $m=1,..,5$.

Impact of the PEF treatment factors on their square model parameters is shown in Fig. 6. The biggest effect was produced by electric field (Fig. 6,a), type of solvent(Fig. 6,c), and treatment duration (Fig. 6,d).

From the obtained datashown in Fig. 6, the optimized PEF parameters according to their effects on oil yield are:

1. Electric fieldhas a monotonously growing effect. A similar dependence was reported in [8] and [20]. From our experiments,the optimal value (maximum value at *a5 in* Fig. 6a*)* is $7 kVcm^{-1}$.

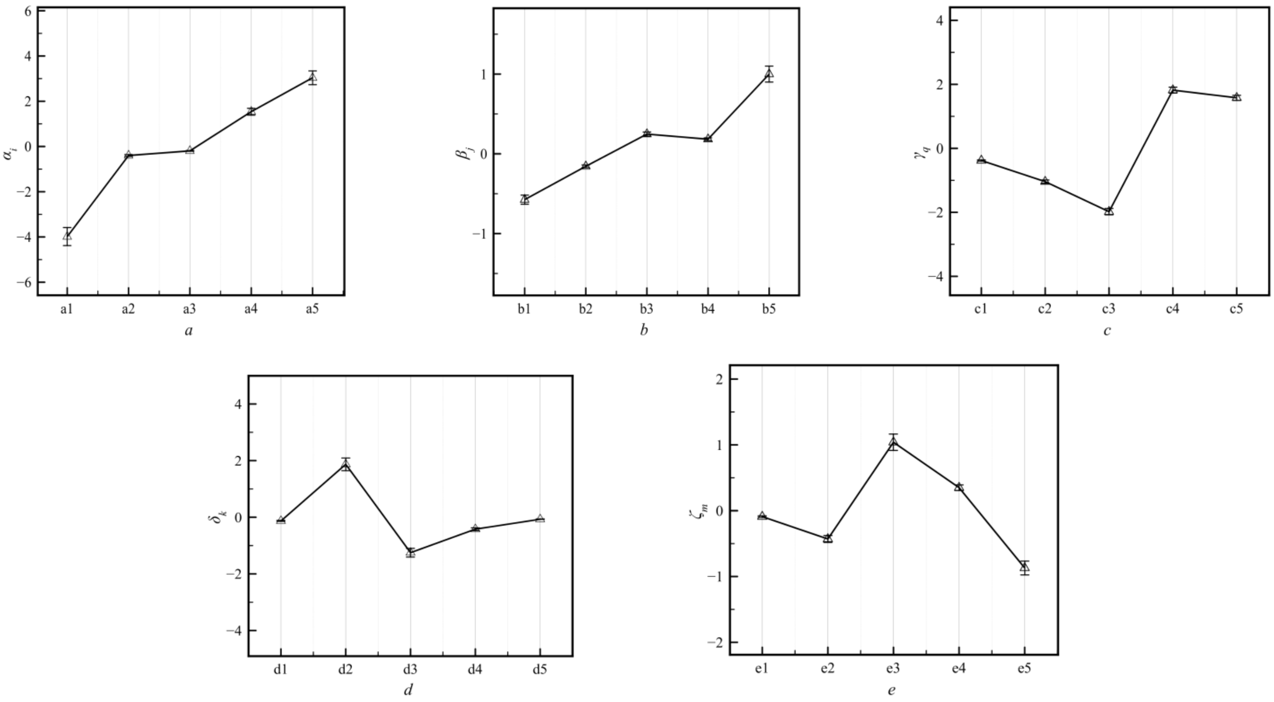

Figure 6.Impact of the PEF factors on the square model parameters

2. Frequencyalso has a monotonously growing effect.However, the impact of frequency is too small tobe taken into account. The maximum value of 15 Hzwas obtained at *b5*,Fig. 6b.
3. The effect of solvent content was found to have amaximum value at *c4*, Fig. 6c for 40 wt% of solvent.
4. Optimal treatment duration is 30 sec (at *d2*, Fig. 6d).
5. Maximum effect value of pulse width (30 µs) was obtained at *e3*, Fig. 6e.

IV. Conclusions

In this work the effect of PEF treatment on oil extraction yield from sunflower seeds was investigated.The optimal PEF parameters were determined, and oil yield for those parameters was measured. Maximum disintegration indexand oil yield of respectively 42.3% and49.11% were achieved for the sunflower seeds PEF treated for 30 sec at electric field of 7.0 $kVcm^{-1}$having frequency of 15 Hz and pulse width of 30 µs.

The results of this study show that treatment with pulsed electrical fieldcould be used as an intensification green technology method to increase oil yield from sunflower seeds.

The obtained results will be used in future in non-thermal food processing technologies for larger scaleoil extraction from sunflower seeds.

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References

[1] List, G. “Sunflower seed and oil”. Lipid Technology, 2014, 26 (1), pp. 24.

[2] Lukomec, V., Krivoshlikov, K. “Russian raw sector of oil industry: situation, perspectives. Eighth international conference “Russian oil and fat complex: new aspects of development”, 2014, pp. 26-29.

[3] E. Koshevoi, Technological equipments of plant oil factories. SPb: GIORD, 2001.

[4] E. Camy, S. Destrac, P. Condoret, J. Stéphane, “Influence of pretreatments for extraction of lipids from yeast by using supercritical carbon dioxide and ethanol as cosolvent”. The Journal of Supercritical Fluids, 2011, vol. 58-1. pp. 68-78.

[5] F. Chemat, M. Vian, G. Cravotto, “Green extraction of natural products: concept and principles”. Int. J. Mol. Sci, 2012, 13, pp. 8615-8627.

[6] N. Lebovka, I. Praporscic, E. Vorobiev, “Effect of moderate thermal and pulsed electric field treatments on textural properties of carrots, potatoes and apples”. Innovative Food Science and Emerging Technologies, 2004 5, pp. 9−16.

[7] M. Fincan, P. Dejmek, “In situ visualization of the effect of a pulsed electric field on plant tissue”. Journal of Food Engineering, 2002, 55, pp. 223−230.

[8] T. Kathiravan, R. Kumar, J. H. Lakshmana, M. R. Kumaraswamy, S. Nadanasabapathi, “Pulsed electric field processing of functional drink based on tender coconut water (Cococus nucifera L.) - nannari (Hemidesmus indicus) blended beverage”, Croat. J. Food Sci. Technol.,2014, 6-2, pp. 84-96.

[9] R. Sarkis, N. Boussetta, I. Tessaro, L. Marczak, E. Vorobiev. “Application of pulsed electric fields and high voltage electrical discharges for oil extraction from sesame seeds ”Journal of Food Engineering, 2015, 153, pp. 20–27.

[10] M. Regitanodarce. “Sunflower-seed oil extraction with ethanol” Journal of the American oil chemists society. 1986, 63-4, pp. 428.

[11] K. Kardjilova, Y, Rangelov, Z. Hlavacova, “Measurement of Electrical Properties of Spelled Grains” Int. J. of App. Sc. and Tech, 2013, vol.3, 7. pp. 118-126.

[12] M. Guderjan, A. Mueller, A. DeArevalo, H. Steinhart, R. Eggers,. and D. Knorr. “Impact of pulsed electric fields on the recovery of oils and phytosterols from corn”. Journal of the Science of Food and Agriculture, 2007, 8, pp. 55-62.

[13] Z. Liu, X. Zeng, D. Sun, Z. Han, R. Aadil, “Synergistic effect of thermal and pulsed electric field (PEF) treatment on the permeability of soya PC and DPPC vesicles” Journal of Food Engineering, 2015, 153, pp.124–131.

[14] M. Guderjan, P. Elez-Martínez, D. Knorr, “Application of pulsed electric fields at oil yield and content of functional food ingredients at the production of rapeseed oil”, Innovative Food Science and Emerging Technologies, 2007, 8, pp. 55–62.

[15] S. Ahnazarova,V. Kafarov, “Methods of experimental optimization in chemical technology”, CA: Moscow, High school, 1985.

[16] I. Shorstkii, “Comparison between denaturated ethanol hexane and absolute ethanol for oil extraction from sunflower seeds” unpublished.

[17] E. Vorobiev,N. Lebovka, “Pulsed electric fields induced effects in plant tissues: fundamental aspects and perspectives of application”. Electrotechnologies for Extraction from Food Plants and Biomaterials. Springer New York, 2008, pp. 39-82.

[18] N. Lebovka, M. Bazhal, E. Vorobiev, “Estimation of characteristic damage time of food materials in pulsedelectric fields”. J Food Eng 2002; 54, pp. 337-346.

[19] M. Brevedan, A. Carelli, G. Crapiste, “Changes in composition and quality of sunflower oils during extraction and degumming” Grasas y Acietes, 2000, Vol.51, 6, pp. 417-423.

[20] N. Boussetta, N. Grimi, E. Vorobiev, "Pulsed Electrical Technologies Assisted Polyphenols Extractionfrom Agricultural Plants and Bioresources: A Review", International Journal of Food Processing Technology, 2015, 2, pp. 1-10.