

Optimization of producing oil and meal from canola seeds using microwave – pulsed electric field pretreatment

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Abstract – In this study, optimization of the extraction of canola seeds oil was investigated using microwave-pulsed electric field seeds pretreatment (MW-PEF) with different MW times (0 to 200 s) and PEF intensities (0 to 5 kV/cm). The seeds oil was then extracted using screw press with different speeds (11 to 57 rpm). Oil extraction efficiency, refractive index, peroxide and phenolic compounds of oil and meal protein were measured. Tocopherols content of the best sample was also measured. The results showed that the peroxide and phenolic compounds increased at higher time, intensity and speed. An increase in the MW time and PEF intensity at first led to an increase in the oil extraction efficiency and meal protein but then both parameters decreased. The efficiency of oil extraction and protein decreased at higher speeds. The refractive index of all samples was 1.475. Gamma tocopherol was predominate one in canola oil and applying the pretreatment led to an increase in the number of total tocopherols. Treating at 1.28 kV/cm for 140.5 s and 28.71 rpm was chosen as the optimum condition with high desirability (0.744).

Keywords: pretreatment / canola / microwave / pulsed electric field / oil

Résumé – Optimisation de la production d'huile et de tourteaux à partir de graines de canola prétraitées par microondes et champ électrique pulsé. Dans cette étude, l'optimisation de l'extraction d'huile de graines de canola a été étudiée en utilisant un prétraitement des graines par micro-ondes et champs électriques pulsés (MO-CEP), avec différentes durées sous MO (0 à 200 s) et différentes intensités de CEP (0 à 5 kV/cm). L'huile de graines a ensuite été extraite à l'aide d'une presse à vis à différentes vitesses (11 à 57 tr/min). L'efficacité de l'extraction de l'huile, l'indice de réfraction, les peroxydes et composés phénoliques de l'huile ainsi que la protéine du tourteau ont été mesurés. La teneur en tocophérols du meilleur échantillon a également été mesurée. Les résultats ont montré que les peroxydes et composés phénoliques augmentaient avec le temps, l'intensité et la vitesse. Une augmentation du temps de MO et de l'intensité des CEP a tout d'abord entraîné une augmentation de l'efficacité d'extraction de l'huile et de la protéine du tourteau, mais a ensuite diminué les deux paramètres. L'efficacité de l'extraction de l'huile et la protéine ont diminué aux vitesses les plus élevées. L'indice de réfraction de tous les échantillons était de 1,475. Le gamma-tocophérol était prédominant dans l'huile de canola et l'application du prétraitement entraînait une augmentation de la concentration en tocophérols totaux. Le traitement à 1,28 kV/cm pour une durée de 140,5 s et à 28,71 tr/min a été choisi comme condition optimale avec une désirabilité élevée (0,744).

Mots clés : prétraitement / canola / micro-ondes / champ électrique pulsé / huile

1 Introduction

The oilseeds are the second important food reserves of the world after cereals and meanwhile, canola is considered as one

of the main oilseeds all over the world (Ashraf and McNeilly, 2004; Azimi *et al.*, 2012). Canola is the modified rapeseed that is the result of genetic manipulations in Brassica napus and Brassica Rapa species. Its extracted oil contained 2% Erucic acid and 30 mg Glucosinolate per one gram of dried meal. In comparison to the other oil seeds, canola should specific

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characteristics such as having higher oil content; hence, this seed is more economic value among the oilseeds and having numerous agricultural benefits. Canola is known as one of the most important oilseeds therefore many researchers studied its properties (Przybylski and Mag, 2011). Varieties of canola can belong to one species of *Matthiola* family including Rapeseed (*Brassica napus* L.), oil Turnip (*B. rapa campestris* L.) and Indian mustard (*B. juncea* L.) (Barthet, 2016). Because of having high amounts of unsaturated fatty acids and low amounts of saturated fatty acids (71% or less), the canola oil is considered as an important and healthy source of edible oil (Gul and Ahmad, 2004; Gerzhova *et al.*, 2016). After soybean and oil palm, canola is the third source for oil production (Rathke *et al.*, 2006). The method of nurturing the rapeseed is one of the effective factors on the seeds function, oil percentage and the quality of its seed. The canola oil is the only edible oil that contains sulphureted fatty acids. The unsaturated fatty acids are formed the main part of fatty acid compounds in canola oil (Ohara *et al.*, 2009). In comparison to the other oil seeds, rapeseed showed more phenolic compounds (Nowak *et al.*, 1992). Sinapic acid and its derivatives, most notable Sinapine, are the most significant of them. Most of the phenolic compounds remain in the meal during the seed pressing, but most abundantly vinylsyringol is founded in crude rapeseed oil (Koski *et al.*, 2003). The lower phenols recovery of the heat-treated seed is related to the incomplete extraction using 70% ethanol due to complexation with heat-denatured protein (Naczka *et al.*, 1992). It was mentioned canolol as the substance isolating from crude canola oil. Canolol shows high antioxidant capacity and anti-mutagenic properties (Koski *et al.*, 2003; Kuwahara *et al.*, 2004; Vuorela *et al.*, 2004, 2005; Wakamatsu *et al.*, 2005). According to Kuwahara *et al.* (2004), its anti-mutagenic potency is higher than that α -tocopherol and flavonoids. In rapeseed, sinapic acid derivatives are the predominant phenolics. Canolol can be produced by decarboxylation of sinapic acid during the press process or roasting of the seeds (Koski *et al.*, 2003; Kuwahara *et al.*, 2004). Thus, the food value of the rapeseed and rapeseed oil may be enhanced by elevating the canolol content through press processing or the roasting of rapeseed before pressing.

Applying microwave (MW) leads to a reduction in the oil extraction process time as well as the energy consumption, for this reason, several studies and researches are performed in the case of using these waves in oil extraction. The kinetics of rapeseed oil and tocopherols extraction was studied during MW treatment. Results indicated that using these waves in each time and temperature increased the process efficiency and Fick's modified diffusion model was the best one to describe the extracting oil process (Sánchez *et al.*, 2017). MW pretreatment was applied in extracting oil from Moringa seeds with supercritical solvent in laboratory scale (Da Porto *et al.*, 2016). The results of this research indicated that the extraction efficiency increased by applying MW and also the use of supercritical solvent caused producing better and higher quality oil in comparison to the oil extracted using Soxhlet.

The pulsed electric field (PEF) process, as a non-thermal method, is widely used to optimize the energy consumption and preserve the qualitative properties of the food products. During recent years, this method is used in industrial scales in biotechnology, pharmacy, making stimulus lasers, cleaning of

Table 1. Experimental levels of each independent variable: MW time (X1), PEF intensity (X2) and rotational speed of screw press (X3).

Number	X1	X2	X3
1	0	0	34
2	200	0	34
3	0	5	34
4	200	5	34
5	0	2.5	11
6	200	2.5	11
7	0	2.5	57
8	200	2.5	57
9	100	0	11
10	100	5	11
11	100	0	57
12	100	5	57
13	100	2.5	34
14	100	2.5	34
15	100	2.5	34
16	100	2.5	34
17	100	2.5	34

metals and polymer materials, producing and processing the foodstuffs such as fruits extract, milk and liquid eggs. This process consists of applying some pulses in short times (about microsecond times) at the strong electric field intensities on the foodstuff placed between two electrodes at room temperature. PEF with high intensity cause the electrical decomposition of cells and increases their permeability (Pourzaki and Mirzaee, 2009; Schroeder *et al.*, 2009). Some researchers studied the effective parameters of the PEF at the time of extracting oil from sunflower seeds. They studied the effect of different parameters such as the PEF intensity, frequency, pulse width, treatment time and also amount of the solvent on the efficiency of oil extraction from sunflower seeds. The results indicated that when the seeds were treated with a PEF (7 kV/cm, 1.5 Hz, 30 μ s [pulse width] for 90 seconds) the efficiency of oil extraction was increased about 9.1%. Eventually, they stated that it's possible to use this process in extracting oil from sunflower seeds in higher scales (Shorstkii *et al.*, 2017). Zeng *et al.* (2010) and Bakhshabadi *et al.* (2018) were some other researchers who had used PEF for oil extraction (Zeng *et al.*, 2010; Bakhshabadi *et al.*, 2018). Researchers showed that application of combined pretreatment of hydrothermal-MW leads to increase in the efficiency of oil extraction (Cortese *et al.*, 2019). In the previous studies, only one pre-treatment was applied individually to enhance the oil extraction while in the current study, two different sequential pre-treatment (MW and PEF) were used to increase the efficiency of canola oil extraction.

2 Materials and methods

2.1 Materials and equipment

The canola seeds (contain 41.3% oil) were provided from the local market of Fars Province (Iran) to perform this

research. Then, the external materials such as weed seeds, sand, and stones were separated and removed from it by hand and the seeds were transferred to Food Industry Laboratory of the Islamic Azad University of Gonbad-e-Kavoos and Modern technologies laboratory of Food Industry Research Institute to perform some experiments. Equipment that was used in this research consists of laboratory sieves, desiccator, laboratory oven (Member, made I Germany), digital balance (Gec Avery, made in England), refractometer (Abbe, Cruise, made in Germany), microwave device (LG, made in South Korea), pulsed electric field device that was made in Food Industry Research Institute of Iran, high performance liquid Chromatography (Knauer, made in Germany), Scanning electron microscope (SEM) (Oxford, England) and laboratory screw press (Kern Kraft, Made in Germany).

2.2 Applying compound pretreatment on the seeds and extracting oil

The canola seeds were affected and treated by MW pretreatment with power of 540 Watt and different process times (0, 100 and 200 s). After MW treatment, the samples were transferred to the water chamber and let it cooled down up to room temperature (25 °C). Then, the seeds were treated by PEF at three intensities (0, 2.5 and 5 kV/cm) with fixed pulse numbers of 30. PEF process was carried out by a unipolar batch PEF generator system, producing logarithmic pulses with 0.5 ms duration. The capacity of process chamber was 4 L. The camber was filed with seeds (0.5 kg) and water (1 kg) as the conductive liquid. The chamber and two parallel electrodes were made of the transparent carbonate (Plexiglas) and stainless steel, respectively. The maximum voltage of instrument was 7 kV and its capacitance was 8 μ F (Guderjan *et al.*, 2005; Uquiche *et al.*, 2008; Bakhshabadi *et al.*, 2017). The moisture content of the seeds homogenously control and reduced to 6% by drying at 50 °C; then, the oil of seeds was extracted using screw press with different speeds (11 to 57 rpm) and various experiments were performed on oils and proteins.

2.3 Calculating the efficiency of oil extraction (oil extraction efficiency)

The amount of extraction efficiency was obtained using equation (1) and with a digital balance based on the introduced method by Bakhshabadi *et al.* (2017). It should be mentioned that to control the effect of moisture content on the efficiency of oil extraction, the seeds had uniform moisture content.

$$\text{Extraction yield(\%)} = \frac{\text{Extracted oil(g)}}{\text{Seeds' weight}} \times 100. \quad (1)$$

2.4 Measure the refractive index

After sedimentation, the oil drop was obtained from the surface to measure the oil refractive index according to the AOCS Cc 7-25 method (1994) using refractometer device at 25 °C (AOCS and Firestone, 1994).

2.5 Determine the peroxide index

The peroxide amount of oils was measured according to AOCS Dd 8-53 method (1994).

2.6 Determine the amount of total phenolic compounds

The amount of total phenolic compounds was determined with the colorimetry method using Folin Ciocalteu's phenol reagent (Bail *et al.*, 2008).

2.7 Measure the number of tocopherols in the optimized and control sample

Determine and measure the number of total tocopherols was performed according to the AOCS Ce 8-89 method (1994) with a high-performance liquid chromatography device (HPLC). For this purpose the 5-60 column (HICHROSORB) with dimensions of 250* 4.5 mm and particle size of 5 μ , with fluorescence detector was used. The mobile phase has selected a combination of Acetonitrile with distilled water at a 95 to 5 ratio and was used. The flow rate of mobile phase was 0.6 mL/min. the number of tocopherols in samples was determined on the basis of the remaining time of tocopherols and also the chromatogram resulted from oil samples. The oil of control sample, without applying any pre-treatments, was extracted using screw press (34 rpm).

2.8 Measure the protein

The amount of nitrogen in the seeds was measured using the fully-automated Kjeldahl device and according to the (Association of Official Analytical Chemists) AOAC 03-990 (2008). After titration, the amount of nitrogen was calculated and the protein amount of the meals was measured using the conversion factor of 6.25 (AOAC, 2008).

2.9 Scanning electron microscopy analysis (SEM)

The scanning electron microscope was used to study the structure of samples (control and treated one). At first, after it was assured that samples are dried, they were glued on the aluminum base using silica glue and then, the bases were coated in a gold coating/sputtering device. At last, imaging from samples was performed in magnification of 1000.

2.10 Statistical analysis

The response surface methodology using the Box-Behnken design was used to evaluate the fixed parameters of study, MW time (X_1), PEF intensity (X_2), rotational speed of screw press (X_3) on the amount of oil extraction efficiency, refractive index, peroxide and phenolic compounds and protein amount of meals the variable parameters (Tab. 1). Using this design and method, all coefficients of the second-order regression model and interaction of factors are measurable and estimable. The most important case in this research is to study the interaction of factors and finding the best condition of the oil

Table 2. Model selection for dependent (response) variables.

Models	Oil extraction yield		Refractive indexes		Peroxide value		Phenolic compounds		Meal protein	
	Sum of squares	Pb>F	Sum of squares	Pb>F	Sum of squares	Pb>F	Sum of squares	Pb>F	Sum of squares	Pb>F
Mean	16 600		36.99		91.45		566 000		15 365	
Linear	443.27	0.052	0.000	1.0000	528	< 0.0001	156 200	< 0.0001	87.21	0.0481
2FI	147.4	0.376	0.000	1.0000	0.020	0.002	12 701	< 0.0001	13.39	0.712
Quadratic	426.93	< 0.0001	0.000	1.0000	0.0003	0.959	6.87	0.272	94.91	< 0.0001
Cubic	0.0029	0.998	0.000	1.0000	0.00002	0.0994	0.00092	0.987	0.24	0.7156
Residue	0.46		0.000		0.006		10		0.67	
Total	17 618.9		36.99		96.17		582 000		15 561.89	

Note: The suggested models are shown on bold highlighting.

extraction process and producing a protein-rich meal from canola seeds, therefore, the response surface statistical design was selected. A quadratic polynomial equation was fitted for each independent variable to evaluate the behavior of response surfaces. Finally, the ASA software was used to compare the samples obtained from optimization pretreatment with control sample for the number of tocopherols and Duncan's multiple range tests were used to compare the data means.

3 Results and discussions

3.1 The influence of studied parameters on the efficiency of oil extraction

As shown in Table 2, the quadratic model was the best model to interpret oil extraction efficiency. Table 3 indicated that all of the studied parameters had a significant effect on oil extraction efficiency ($P < 0.001$). As represented in Figure 1, the oil extraction efficiency increased at first and then reduced by an increase in the MW time and PEF intensity. Also, the figures obtained from scanning electron microscope (SEM) (Fig. 2) confirmed that using this pretreatment lead to texture destruction and create more pores and outlets resulting in better oil extraction from seeds. Also, the results of this part indicated that the oil extraction efficiency decreased with an increase in the rotational speed of screw press because of a decrease in the pressure on seeds. The MW causes to evaporate water from plant structure and pressure increase in the internal environment and this case can lead to material decomposition, membrane disintegration, increase in the oil extraction efficiency using press and oil passing from the cell membrane (Aguilera and Stanley, 1999). Researchers reported that the efficiency of extraction process can be increased by an increase in the MW time (Oomah *et al.*, 1998; Azadmard-Damirchi *et al.*, 2010; Sánchez *et al.*, 2017). Also, the results of this part indicated that the efficiency of the extraction process decreased with an increase in the rotational speed of press because reducing in the pressure on the samples (Deli *et al.*, 2011). An increase in the oil extraction efficiency with increase in the PEF intensity can be related to an increase in the membrane degradability index (Sarkis *et al.*, 2015). The oil extraction efficiency was decreased with an excessive increase in the MW time and PEF intensity, probably because of more degradation in the internal structure of seeds and closure of oil outlet. It was

reported that heat treatment with micronutrients results in more efficient extraction of the seed compounds by reducing the moisture content, enzymatic inactivation, or changes in lipid structure in soybean pellets (List *et al.*, 1990). Pradhan *et al.* (2011) investigated the effect of baking process and moisture content on some oil properties of peeled Jatropha seeds. The results showed that the oil extraction efficiency reduced because of using the high temperatures and also producing an elastic state by increase in the moisture content of the kernels. Baking at 110 °C for 10 min was chosen as optimum condition. The increase in temperature, due to the reduction in viscosity of the oil as well as the cell wall degradation, speeds up the extraction rate, increase the oil extraction and reduce the oil remaining in the meal. In high protein content seed, the use of high temperatures reduces the lubrication efficiency due to the excessive alteration in protein structure (Pradhan *et al.*, 2011).

The following model (Eq. (2)) represents the linear relationship between the extraction efficiency and independent variable:

$$y = +38.40 + 2.42X_1 - 3.78X_2 - 5.94X_3 - 1.87X_1X_2 + 5.09X_1X_3 - 2.73X_2X_3 - 5.52X_1^2 - 7.65X_2^2 - 2.02X_3^2. \quad (2)$$

3.2 The influence of operational parameters on the refractive index

All of the test variables (MW time, PEF intensity and the rotational speed of screw press) showed no effect on the oil refractive index. In this research, the refractive index of oil resulted from canola seeds was obtained equal to 1.475 (Fig. 3). The model that is related to the fitted data refractive index for samples is presented as equation (3):

$$y = +1.48. \quad (3)$$

The results of this research conformed to the researchers who studied the physicochemical characteristics of orange seed oil that is extracted with different methods and stated that new methods of extraction in comparison to common methods can't change some of the physical characteristics of oil such as refractive index (Gorji *et al.*, 2016). On the other hand, results

Table 3. Analysis of variance for determined parameters in oil extraction by pretreatment of microwave-pulsed electric field.

Source	Oil extraction yield			Refractive indexes			Peroxide value			Phenolic compounds			Meal protien		
	Sum of squares	F-value	Pb > F	Sum of squares	F-value	Pb > F	Sum of squares	F-value	Pb > F	Sum of squares	F-value	Pb > F	Sum of squares	F-value	Pb > F
Model	1017.6	1709.8	< 0.0001	0.00	1323.6	< 0.0001	5.30	16681	< 0.0001	16890	16681	< 0.0001	195.51	167.7	< 0.0001
X ₁	46.75	707.0	< 0.0001	0.00	4000.6	< 0.0001	2.67	60933	< 0.0001	102800	60933	< 0.0001	0.12	0.9	0.368
X ₂	114.01	1723.9	< 0.0001	0.00	2692.3	< 0.0001	1.80	7550	< 0.0001	12740	7550	< 0.0001	19.75	152.5	< 0.0001
X ₃	282.51	4272.3	< 0.0001	0.00	1218.8	< 0.0001	0.81	24077	< 0.0001	40631	24077	< 0.0001	67.34	519.8	< 0.0001
X ₁ ²	128.3	1940.1	< 0.0001										31.29	241.6	< 0.0001
X ₂ ²	246.7	3731.1	< 0.0001										24.13	186.2	< 0.0001
X ₃ ²	17.18	259.8	< 0.0001										29.54	228.0	< 0.0001
X ₁ X ₂	14.06	212.6	< 0.0001		0.04	0.850	0.00025	5424	< 0.0001	9153	5424	< 0.0001	3.24	25.0	0.001
X ₁ X ₃	103.63	1567.1	< 0.0001		27.33	0.004	0.018	1763	< 0.0001	2975	1763	< 0.0001	1.42	10.93	0.013
X ₂ X ₃	29.7	449.2	< 0.0001		2.4	0.152	0.0016	339	< 0.0001	571	339	< 0.0001	8.73	67.41	< 0.0001
Residual	0.46			0.000			0.0067			16.87			0.91		
Pure error	0.46			0.000			0.006			10			0.67		
Cor total	1018.05			0.000			5.30			168900			196.42		
R-squared	0.99			0			0.99			0.98			0.99		
Adj R-squared	0.99			0			0.99			0.96			0.98		

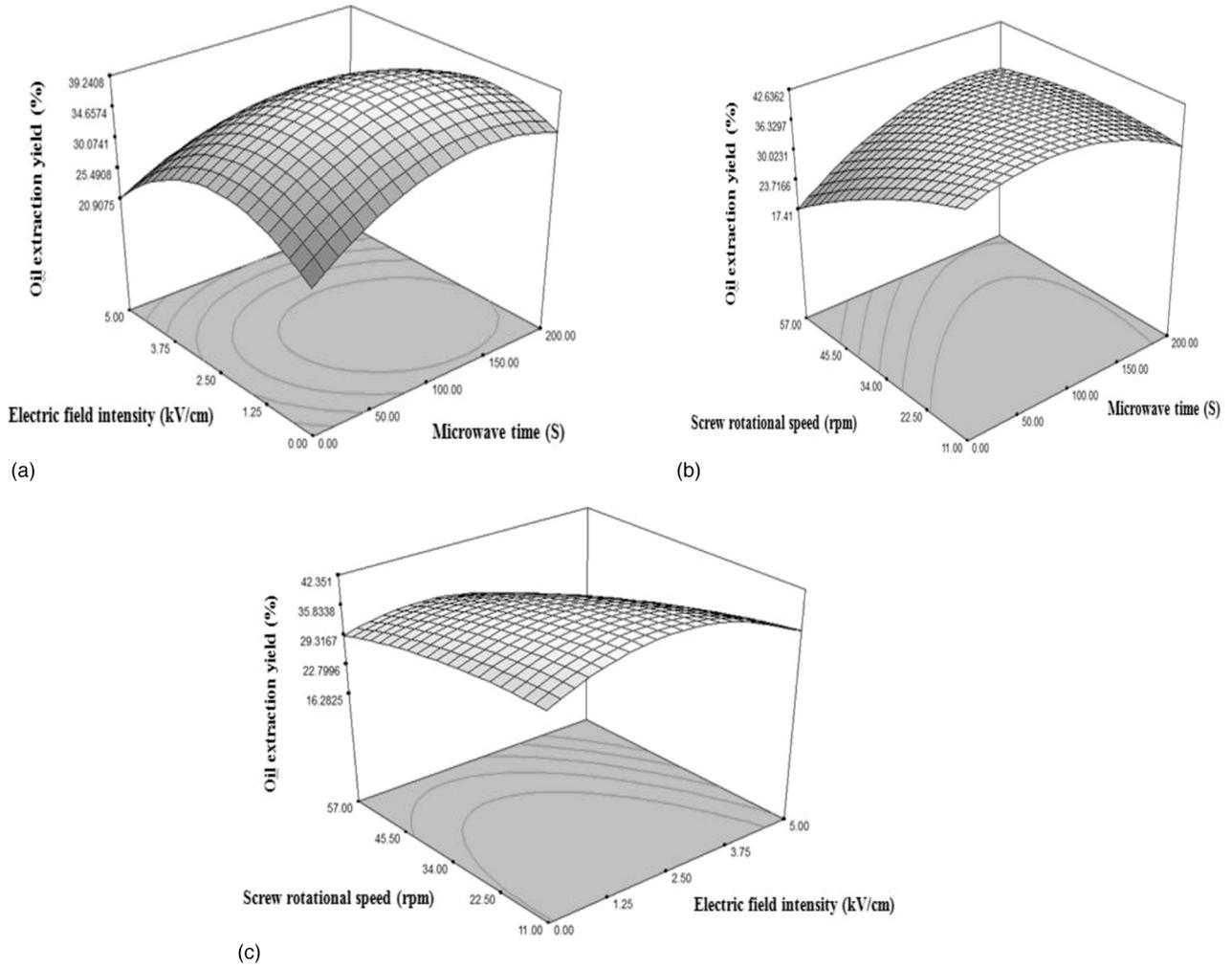


Fig. 1. Three-dimensional diagrams: the influence of (a) microwave time and electric field intensity, (b) microwave time and the rotational speed of screw press and (c) intensity of electric field and rotational speed of screw press on the oils extraction yield.

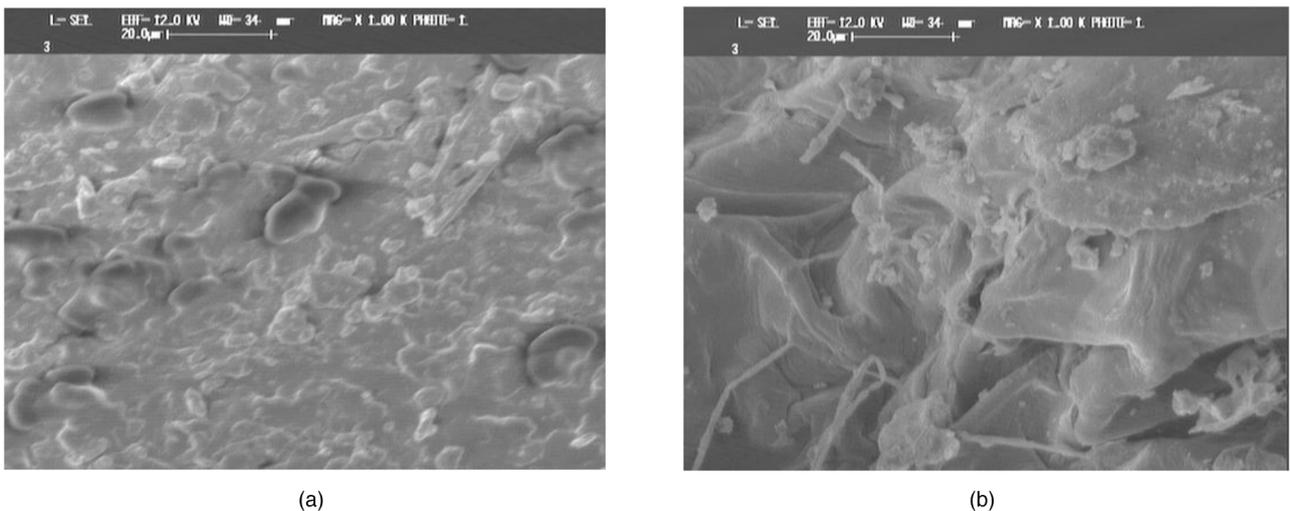


Fig. 2. SEM diagrams from (a) control sample (without treatment) and (b) treatment sample.

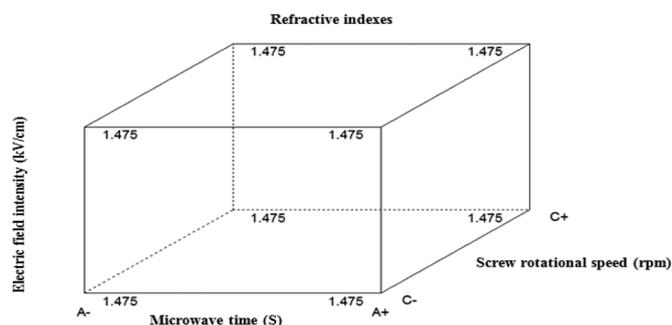


Fig. 3. Cube diagram of changes in the amount of oils refractive index under the influence of microwave time, electric field intensity, and the rotational speed of screw press.

of Uquiche *et al.* (2008) and Tale Masouleh *et al.* (2015) confirmed these results, too (Uquiche *et al.*, 2008; Tale Masouleh *et al.*, 2015). On the other hand, lack of variation and change in the refractive index of oils obtained from this process can be attributed to the similarity of fatty acid profiles in the non-treated and treated samples.

3.3 The influence of understudied fixed variables on the amount of peroxide in oils

According to Table 2, the best-selected model for fitting the data obtained from samples peroxide was a 2FI model that on the basis of that the fitted model for these data was equation (4):

$$y = +2.32 + 0.58X_1 + 0.47X_2 + 0.32X_3 + 0.00025X_1X_2 + 0.068X_1X_3 + 0.020X_2X_3. \quad (4)$$

From the above-mentioned model and also number F, it can be found that the linear parameter of MW time and after that the linear parameter of PEF intensity has the most influence on the peroxide amount of oils. The variance analysis (Tab. 3) indicated that the linear parameters and also mutual parameters of MW time with the rotational speed of the screw press had a significant influence in this model. The relationship between studied parameters (Fig. 4) clearly indicated that the peroxide amount increased with an increase in the MW time, PEF intensity and the rotational speed of screw press. The reason for the increase in the peroxide amount can be attributed to more oxidation of fatty acids that will become more with temperature increase (Guderjan *et al.*, 2007). The results of this part conformed to results and findings of other researchers who had stated that oils peroxide index will increase with rising in the temperature (Valentov *et al.*, 2000; Hassanein *et al.*, 2003; Zeng *et al.*, 2010). These researchers stated that conditions and types of applied operations on the oilseeds had a significant impact on the oil peroxide amount. It was also mentioned that oxidation of compounds such as phospholipids and photo-oxidation phenomena also plays an important role in the highness of peroxide amount in oil extraction.

3.4 The influence of operational parameters on the phenolic compounds

Studying the changes of the total phenolic compounds in canola oil indicated that MW time had a positive effect on the

content of total phenolic compounds; it means that with an increase in the MW time, the amount of these compounds significantly increased. Also, an increase in the PEF and the rotational speed of press had a similar trend on this characteristic (Fig. 5). The model that is related to the fitted data for total phenolic compounds is as equation (5):

$$y = +577.02 + 113.37X_1 + 39.91X_2 + 71.27X_3 - 47.84X_1X_2 + 27.28X_1X_3 + 11.96X_2X_3. \quad (5)$$

The natural compounds (phenols) can increase the shelf life of food materials through preventing the growth of pathogenic microorganisms that corrupt the food and also through protecting food materials from damage and losses resulted from oxidative stress (Padmashree *et al.*, 2007). Phenolic compounds are the large group of secondary herbal metabolites which their antioxidant ability results from the existence of hydroxyl groups in their structure (Muanda *et al.*, 2011). The principles of heating using MW energy are related to the direct influences of these waves on the molecules with mechanisms of dipolar rotation and ionic conduction. The polar molecules such as phenolic compounds and ionic solutions absorb too much MW energy because of having a dipole moment that results to increase in temperature and quick reaction completion and this case will lead to enter more of these compounds into the oil (Proestos and Komaitis, 2008). With the increase in the PEF intensity and the rotational speed of press because of more releasing and entering of these compounds into the oil the amount of these compounds was increased. The results of this part are conformed to the results reported by other researchers (Boussetta *et al.*, 2014; Jiao *et al.*, 2014). The main phenolic compounds in crude post-expelled rapeseed oil were vinylsyringol (a decarboxylation product of sinapic acid) followed by sinapine and sinapic acid. The content of phenolics decreased during processing which is in agreement with the found results during studying the call (vinylsyringol) in crude rapeseed oil (Koski *et al.*, 2003; Kuwahara *et al.*, 2004). The pre-expelled rapeseed oil contained only a small amount of phenolics indicating that higher temperature (< 100 °C) and pressure are necessary for release of the phenolics into rapeseed oil.

3.5 The influence of studied parameters on the protein amount of meal

The optimized use of a protein that is required for the body is dependent on the digestibility and pattern of essential amino acids in food sources that are provided for the body. Hence, determining the amount of protein and evaluating the used food materials, are necessary for food programming and feeding to provide humans biological needs (Taghizadeh *et al.*, 2007). On the other hand, pricing the meals in oil extraction plants is on the base of the amount of their protein and humidity (Rostami *et al.*, 2014). Table 3 indicated that all of the studied parameters except MW time had significant influence of the protein amount of meal ($P < 0.001$) and on the other hand, regarding the Table 2, it was characterized that the best model for interpreting and determine the amount of protein in the meal was the quadratic model. As it is clear in Figure 6, similar to the oil extraction efficiency, with an increase in the

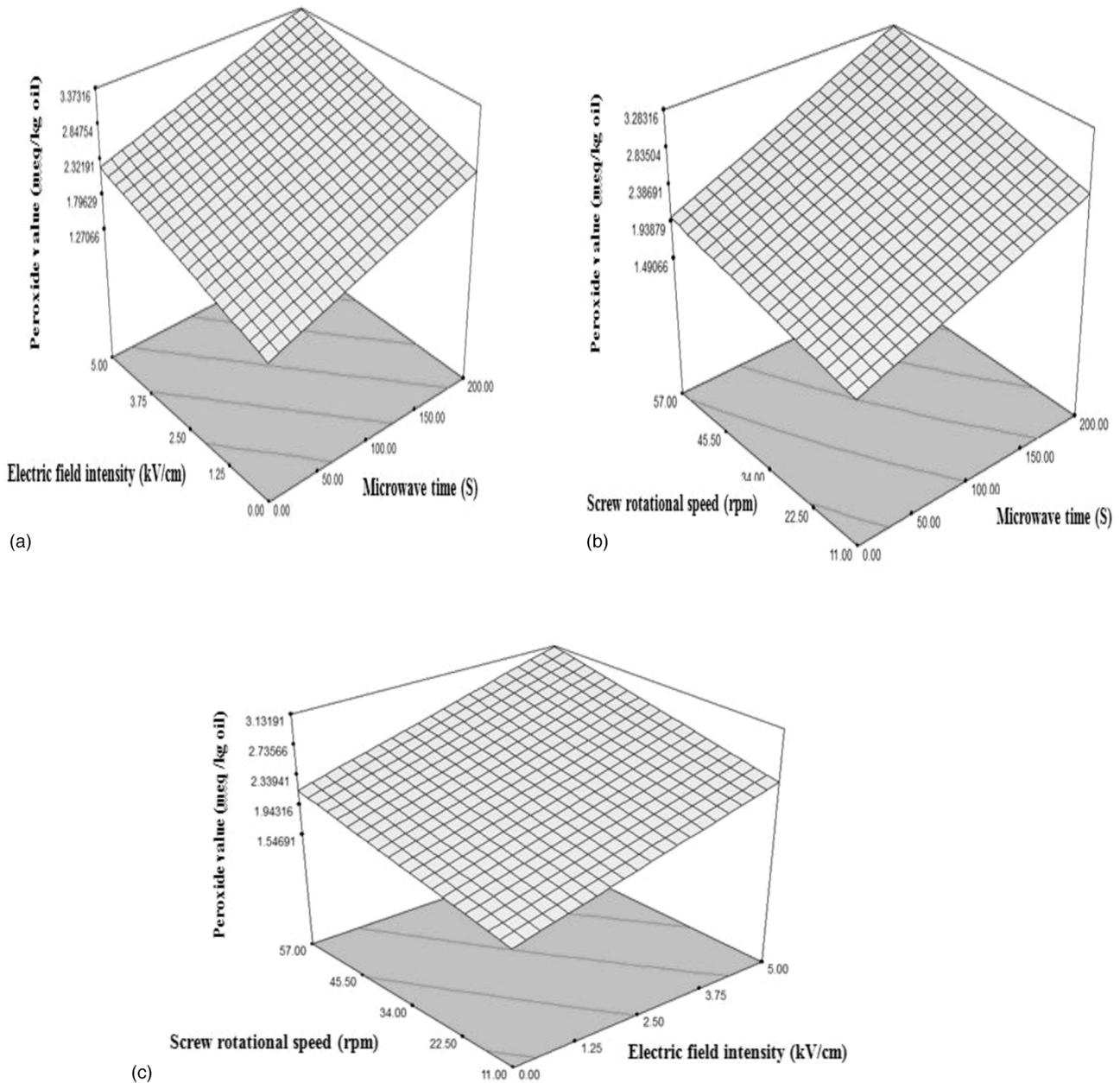


Fig. 4. Three-dimensional diagrams: the influence of (a) microwave time and electric field intensity, (b) microwave time and rotational speed of screw press and (c) intensity of electric field and rotational speed of screw press on the oils peroxide amount.

MW time and PEF intensity, the amount of protein increased at first and decreased then. Also, the results of this part indicated that with an increase in the rotational speed of the screw press the amount of protein in the meal decreased. Applying MW caused an increase in the amount of dissolved protein in the meal. The results of this part confirmed the results of studies performed by others (Choi *et al.*, 2006). The amount of protein in the meal decreased with an increase in the rotational speed of the press, because of protein denaturation and also remaining more oil in the meal. Also, it was indicated that using a PEF caused an increase in the amount of extracted protein (Sarkis *et al.*, 2015). The results of this part were conformed to the results of Bakhshabadi *et al.* (2018). The final model that is presented in the following for the protein amount

of meal and also the F-number indicated more influence of linear variable of rotational speed in screw press on this factor (Eq. (6)):

$$y = +32.72 + 0.12X_1 - 1.57X_2 - 2.90X_3 - 0.90X_1X_2 + 0.59X_1X_3 - 1.48X_2X_3 - 2.73X_1^2 - 2.39X_2^2 - 2.65X_3^2. \quad (6)$$

3.6 Optimization of oil extraction from canola

To find the best condition of extracting oil from canola seeds using MW-PEF pretreatment, regarding the MW time

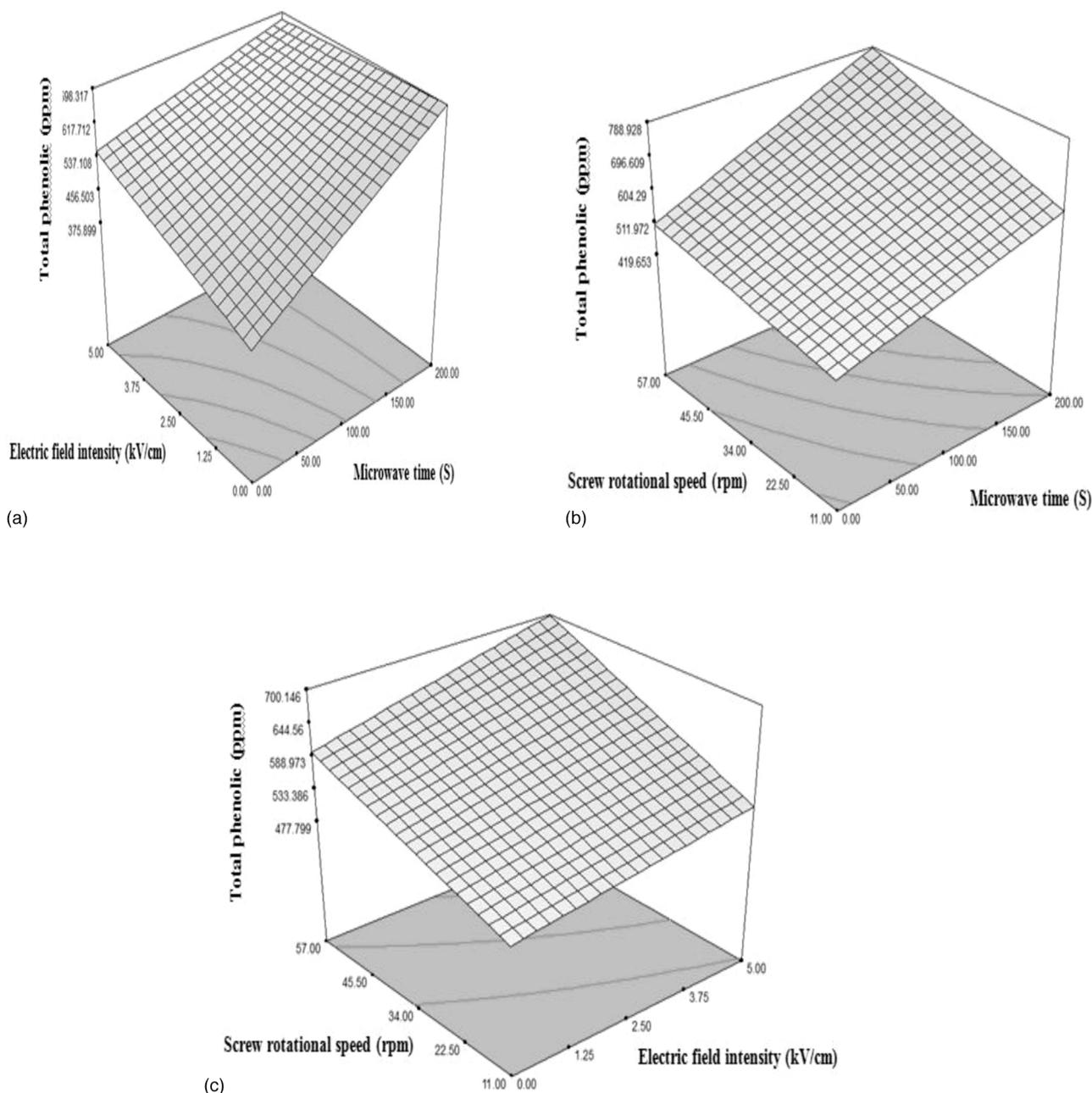


Fig. 5. Three-dimensional diagrams: the influence of (a) microwave time and electric field intensity, (b) microwave time and the rotational speed of screw press and (c) electric field intensity and the rotational speed of screw press on the oils phenolic compounds.

that was set between 0 to 200 seconds, and PEF intensity that was set in the range of 0 to 5 kV/cm and rotational speed of screw press that was set between 11 to 57 rpm, the oil extraction process in the above mentioned conditions was optimized to reach the maximum amount of extraction efficiency, protein in meal and total phenolic compounds and minimizing the amount of peroxide. The results indicated that to reach the mentioned aims, its necessary to set the MW time to 140.51 seconds, the PEF intensity to 1.28 kV/cm and the rotational speed of screw press to 28.71 rpm to reach the desirability of 0.744 in the above-mentioned conditions.

3.7 The influence of MW-PEF on the number of total tocopherols in oil

The mean comparison of data obtained from tocopherols amount indicated that gama tocopherol was the most tocopherol in the oil of both optimized and control samples that was after the alpha-tocopherol (Tab. 4). As it is clear, applying the pretreatment led to increasing in the number of total tocopherols of canola oil and this effect is especially seen in the number of delta tocopherols because this tocopherol is not seen and found in the oil that was extracted without using

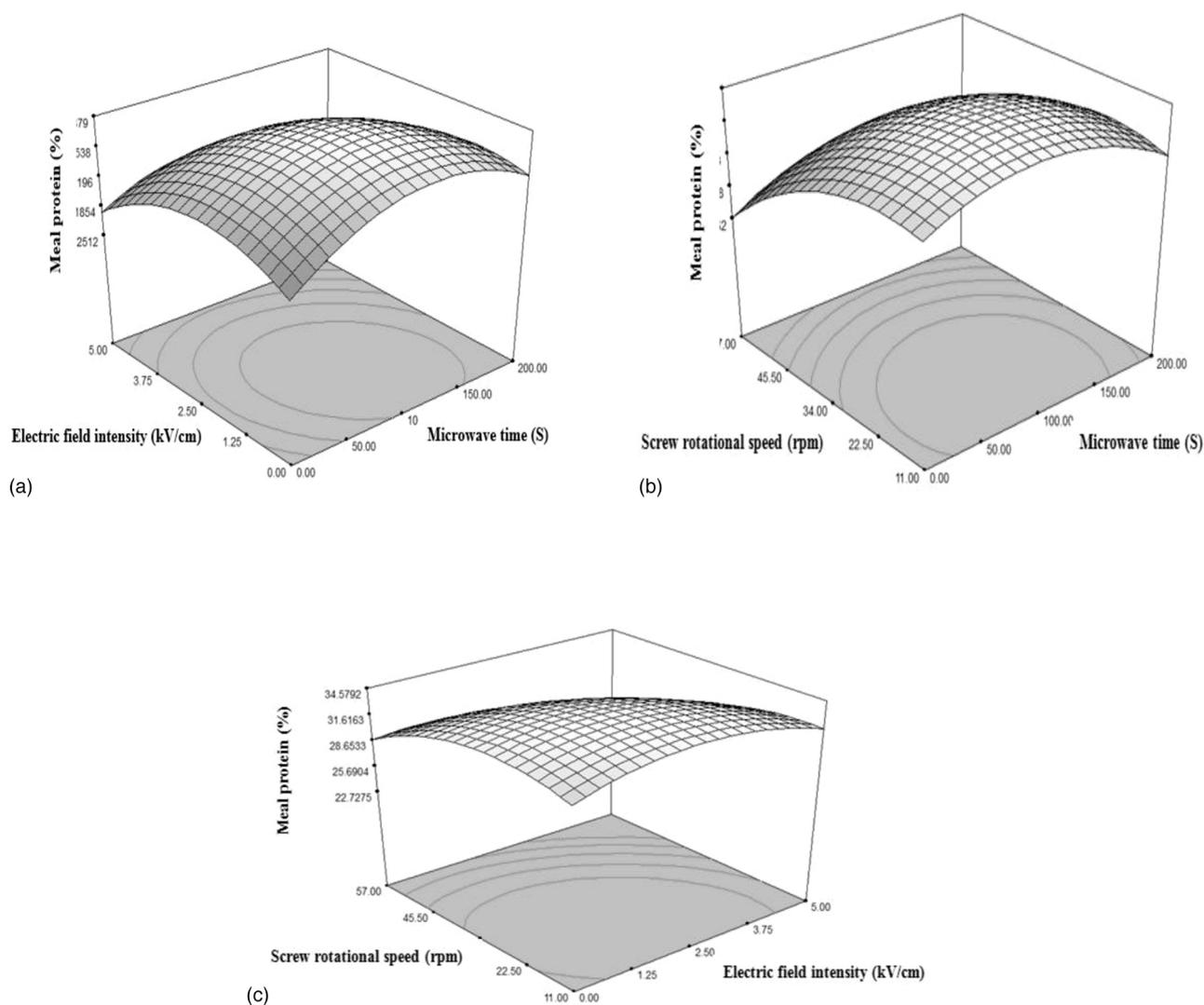


Fig. 6. Three-dimensional diagrams: the influence of (a) microwave time and electric field intensity, (b) microwave time and the rotational speed of screw press and (c) electric field intensity and the rotational speed of screw press on the protein amount of meal.

Table 4. Tocopherols of canola seeds oil achieved by different pretreatment.

Tocopherols type	Standard	Optimized sample
Alpha tocopherols (ppm)	367.3 ± 1.94 ^{aB}	351.02 ± 25.80 ^{aB}
Delta tocopherols (ppm)	0.00 ^{bC} ± 0.00 ^{bC}	30.07 ± 0.24 ^{aC}
Gama tocopherols (ppm)	602.05 ± 4.80 ^{aA}	644.52 ± 1.02 ^{aA}
Total tocopherols (ppm)	969.78 ± 6.74	1025.61 ± 27.06

The similar capital and small letter in each column and row respectively demonstrating the non-significant difference in the confidence level of 0.05.

pretreatment. Applying the combined pretreatment during the oil extraction causes more tocopherols to enter the oil because of a decrease in the reaction between antioxidant compounds with polysaccharides, proteins, and peptides of seed (Bakhshabadi *et al.*, 2018). The results of this part confirmed

with the findings in other studies (Wiktor *et al.*, 2015). It was reported an increase in the amount of vitamin E in the grape oil using MW pretreatment, and the most amount of tocopherol was obtained during 9 min of treatment (Oomah *et al.*, 1998). Studying and considering the studies of other researchers indicated that the amount of alpha-tocopherol, gama tocopherol, and delta tocopherols about 300, 400, and 15 ppm, respectively (Ahmed *et al.*, 2005; Guderjan *et al.*, 2007). This effect is also confirmed by other studies reporting no loss in tocopherols was observed during the thermal processing of seeds and explained this as being due to the protective activity of canolol (Wakamatsu *et al.*, 2005; Spielmeier *et al.*, 2009). It was suggested that formation of canolol during roasting can protect the tocopherols against degradation at higher temperatures (Thiyam-Holländer *et al.*, 2012). Roasting of different rapeseed varieties (typical high linolenic rapeseed variety “Brandy” and “PR46W20”; high oleic acid rapeseed HO PN1414) reflected that the content of canolol, as well as γ -T

and PC-8, are highest during roasting at 180 °C. This showed a synergistic relationship between the content of canolol and the tocopherols (Siger *et al.*, 2015; Gracka *et al.*, 2016).

4 Conclusion

The results of this research indicated that with an increase in the MW time, PEF intensity and rotational speed of screw press the amount of peroxide and phenolic compounds of samples increased, while the efficiency of oil extraction and protein of meal increased at first and decreased then. The amount of refractive index of oils was not influenced by the pretreatment conditions. The report obtained from high-performance liquid chromatography device indicated that gamma tocopherol was the dominant tocopherol among the canola oil tocopherols and applying pretreatment led to an increase in the number of total tocopherols of oil. At last, it can be stated that applying the MW-PEF can be presented and determined as an appropriate pretreatment in the oil extraction industry.

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