Optimization of microwave assisted mechanical extraction of oil from canola seeds by using Response Surface Methodology Patil Aniket^{*}, Singh A K a

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Abstract: Present investigation was undertaken with the overall objective of optimizing the microwave assisted pressing parameters, i.e. microwave time, moisture content, heating temperature and heating time for increasing the oil recovery from canola seeds of GSC-6 variety. Response surface methodology was adopted in the experimental design and Box Bhenken design of four variables was chosen. The range of parameters for the experiments was microwave time (2-4 min), moisture content (5%-15%), heating temperature (60-1000°C) and heating time (4-6 min) and single chamber oil expeller was used for oil expelling. The effect of independent variables, i.e. moisture content, microwave duration and heating temperature on oil yield and residual oil in cake were found significant, however, the effect of the heating time was found non-significant. Microwave time, moisture content and heating temperature affected the acid value and the peroxide value of the oil. Heating temperature affected the viscosity of the oil.

Keywords: Box Bhenken design, microwave treatment, canola seeds and oil recovery

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1 Introduction

Canola is an important oilseed crop in many countries and is considered to be the second most abundant source of edible oil in the world. Worldwide canola production is predicted to exceed 15 million tonnes by 2015 (Tan et al., 2011). Canola quality varieties are commonly developed from either of the two species, i.e. *Brassica napus* and *Brassica campestris*. With the development of canola cultivars, the use of rapeseed oil has increased in major producing countries including Canada. The characteristics of canola include higher yield, relatively shorter duration of the crop, a healthy cooking medium, and livestock feed as oil meal contains less than 30 micromoles glucosinolates per gram of defatted meal (Kumar et al., 2009).

There are two main processes commercially available for defattening and deoiling plant products: extraction and

pressing (Terigar et al., 2011). The methods of extraction employed depend on the type of seed, the seed characteristics and the oil content of the seed (Lawson et al., 2010). Mechanical pressing is the most popular method of oil separation from vegetable oilseeds in the world. The safety and simplicity of the mechanical pressing is advantageous over the solvent extraction A chemical free protein rich meal is equipment. obtained by mechanical presses unlike the solvent extraction method (Singh and Bargale, 2000). To increase the oil extraction, pretreatments are being used of which two techniques i.e. microwave and enzyme pretreatments have been observed to increase the oil yield as well as maintain the quality of oil (Uquiche et al., 2008; Azadmard-Damirchi et al., 2010; Elham et al., 2012).

Microwave energy is a superior alternative to several other thermal treatments. Microwaves are non-ionizing electromagnetic waves of frequency between 300 MHz to 300 GHz with positioning in between the X-ray and infrared rays in the electromagnetic spectrum (Elham et al., 2012). The heat production is mainly due to dipole excitation and ion-migration. Friction energy is

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produced as a result of the orientation of the dipoles in the altering electromagnetic field (El-Badrawy et al., 2007). The microwave heating rate is influenced by many factors such as microwave power level, frequency, initial temperature, dielectric properties of the material, and design of microwave applicator; with the exception of dielectric properties (Terigar et al., 2011). Microwave pretreatment improves oil extraction yield and quality, direct extraction capability, lower energy consumption, faster processing time and reduced solvent levels compared with conventional methods (Azadmard-Damirchi et al., 2011).

The present study was aimed to study the effect of microwave duration, moisture content, heating temperature and heating time on oil recovery and to use RSM for further optimization to enhance the yield using the influential process variables from Canola seeds of variety GSC-6.

2 Materials and methods

Canola seeds: Seeds of commercially available canola variety (GSC-6) were procured from Punjab Agricultural University Farms in India. The seeds were stored in polyethylene bags until analysis.

2.1 Proximate analysis

The canola seeds were analyzed for fat, protein, fiber and ash contents. The fat, protein, fiber and ash contents were determined according to the AOAC official method (AOAC, 2000).

(i) *Fat content:* 5 g sample was placed in thimble and oil was extracted in a pre-weighed beaker using petroleum ether in Soxhlet apparatus. The beaker was then heated to evaporate petroleum ether. Final weight of the beaker was taken and used for the estimation of crude fat content of sample.

(ii) *Protein contents*: Protein content was determined by available nitrogen in the sample as shown in following Figure by Micro Kjeldhal method. One gram sample was digested in 25 ml of sulphuric acid (H2SO4) at 420 °C using 5 g K2SO4 + 0.5 g CuSO4 as

catalyst mixture. Digested sample was cooled and 20 ml of deionised water was added to the sample. After adding 25 ml NaOH (40%), the sample was then distilled and the ammonia liberated was collected in boric acid with five to seven drops of indicator solution and then titrated with standard acid (0.1 N HCl) to estimate the protein content.

(iii) Ash Content: Weigh about 5 g sample into the crucible and burnt on the hot plate. Place the crucible and lid in the furnace at $550 \,^{\circ}$ for 6 h to ensure that impurities on the surface of crucible are burned off. Cool the crucible in the desiccator (30 min). After cooling the crucibles to room temperature, the residue left (ash) in crucibles were weighed.

(iv) *Fiber Content*: Two grams of moisture and fat free sample was first digested with 150 ml boiling 1.25% sulphuric acid solution for 30 min. After acid digestion the mixture was filtered through muslin cloth and washing of residue with hot water was carried out to remove traces of acid. Then alkali digestion was performed with 150 ml of 1.25% sodium hydroxide solution for 30 min. Again the mixture was filtered through muslin cloth and then the residue was washed with hot water till free from alkali; dried overnight at 110 °C and weighed. The crucible was heated in the muffle furnace at 600 °C for 2 h. Then it was allowed to cool and weighed again.

2.2 Microwave roasting of canola seeds:

Seeds placed in petri dishes (100 g in each petri dish) were roasted in a consumer-model microwave oven at a frequency of 2450 MHz (900W) for 2, 3 and 4 min. After roasting, canola seeds were allowed to cool to ambient temperature and thoroughly mixed prior to oil extraction.

2.3 Oil extraction by pressing

After microwave roasting, thoroughly canola oil was obtained by pressing 150 g of canola seeds with a laboratory screw press (Komet, IBG Monforts and Germany). The percent yield of the oil recovered was determined by employing the use of the following Equation 1 (Olaniyan, 2007):

The percent oil yield =
$$\frac{W_{OE}}{W_s} \times 100$$
 (1)

Where,

 W_{OE} = weight of oil expressed, g;

 W_S = weight of sample before expression, g.

The residual oil in the pressed cake was obtained by Soxhlet extraction.

2.4 Experimental design and statistical analysis

The four-level Box-Behnken design with four independent variables (moisture content, microwave

duration, heating temperature and heating time) was applied for responses (oil yield, residual oil in cake, acid value, peroxide value, density and viscosity) functions fitting. This design was selected due to the small number of experiments required to estimate complex response functions. For the four-level three-factorial Box-Behnken experimental design, a total of 29 experimental runs are necessary. The uncoded and coded independent variables and experimental design are listed in Table 1. Box-Behnken design allows on the efficient estimation of the first and second order coefficients.

Exp	Coded level				Independent variables			
	Microwave, s	Moisture content, %	Time, min	Heating Temp., ⁰ C	Microwave, s	Moisture content, %	Time, min	Heating Temp., ⁰ C
1	0	1	0	-1	180	15	5	60
2	1	0	-1	0	240	10	4	80
3	0	1	0	1	180	15	5	100
4	-1	-1	0	0	120	5	5	80
5	-1	0	0	1	120	10	5	100
6	0	-1	0	-1	180	5	5	60
7	1	0	1	0	240	10	6	80
8	1	0	0	-1	240	10	5	60
9	0	0	0	0	180	10	5	80
10	0	0	-1	1	180	10	4	100
11	0	0	1	1	180	10	6	100
12	0	-1	1	0	180	5	6	80
13	-1	0	0	-1	120	10	5	60
14	1	0	0	1	240	10	5	100
15	0	0	0	0	180	10	5	80
16	1	1	0	0	240	15	5	80
17	0	0	-1	-1	180	10	4	60
18	-1	0	1	0	120	10	6	80
19	-1	1	0	0	120	15	5	80
20	1	-1	0	0	240	5	5	80
21	0	-1	0	1	180	5	5	100
22	0	0	0	0	180	10	5	80
23	0	0	1	-1	180	10	6	60
24	0	0	0	0	180	10	5	80
25	0	0	0	0	180	10	5	80
26	0	1	1	0	180	15	6	80
27	0	-1	-1	0	180	5	4	80
28	-1	0	-1	0	120	10	4	80

Table 1 Three-level Box-Behnken design with coded and actual variable

Response surface methodology (RSM) was used to study the simultaneous effects of four experimental factors (microwave time, moisture content, heating temperature and heating time) on oil yield, residual oil in cake and quality parameters of the pressed canola oil and to find optimum conditions for a multivariable system. RSM allowed the development of a predictive mathematical model based on the experimental data, which can be employed for interpolation. Design expert was used for the optimization and for plotting the contour maps.

2.5 Oil analysis

Determination of density, acid value, FFA, and peroxide value of the extracted oil was carried out according to the standard AOAC (1980) methods. Viscosity of oil samples was determined by using Bohlin CVO 100 Rheometer

3 Results and discussion

3.1 Proximate analysis

Results of the proximate analysis of the control sample and microwaved canola seeds are shown in Table 2. The petroleum ether extracted oil contents of non-microwaved canola oilseeds of variety GSC-6 was 33.8%. By microwave treating seeds for 120 s, 180 s and 240 s, the oil content of increased significantly (P < 0.05). The moisture contents of the control canola oilseeds of were 8.0%. Contents of protein, fiber, and ash were 19.60%, 11.50% and 4.13% respectively. Microwave treatment of the seeds did not affect the fiber, ash, and protein contents significantly (P > 0.05).

Tε	ıble	2	Pı	roximate	anal	lvsis	of	roasted	/unr	oasted	canola	oilseeds*

Contents	Control	Microwaves				
	connor	120s	180s	240s		
Fat	$33.80 \pm 0.52^{\circ}$	35.06 ± 0.70^{bc}	36.53 ± 0.80^{a}	34.80 ± 0.40^{bc}		
Protein	19.59 ± 0.20^{a}	19.64 ± 0.23^{a}	19.37 ± 0.39^{a}	19.68 ± 0.18^{a}		
Fiber	11.50 ± 0.5^{a}	12.33 ± 0.57^{a}	12.97 ± 0.50^{a}	12.50 ± 0.50^{a}		
Ash	4.13 ± 0.50^{a}	4.20 ± 0.40^{a}	3.66 ± 0.46^{a}	3.73 ± 0.41^{a}		

Note: *Values are means \pm SD, calculated as percentage for of duplicate samples analyzed individually in triplicate. Mean values in the same row followed by the same superscript letters are not significantly different (P > 0.05)

The longer the roasting time, the greater was the loss in weight of the seeds. This loss in weight may reflect total volatile substances, but it was considered to be mostly due to the loss of moisture. This trend agreed with the findings of Anjum et al. (2006); Yoshida et al. (2001); Yoshida and Kojimoto (1994) who reported the loss in weight of sunflower seeds and sesame seeds.

3.2 Oil yield and residual oil yield

The oil yield and residual oil in cake of the microwave treated canola are listed in Table 3. Oil yield and residual oil (%) results ranged between 15.45% to 36% and 1.05% to 6.8%, respectively. The highest oil yield (36%) was obtained from oilseeds pre-treated with microwave for 180 s, moisture content 5%, heating temperature 80 °C and 4 min heating time. Whereas, the lowest oil yield (15.45%) was obtained at 240 s

microwave treatment, 10% moisture, 100 °C heating temperature and 5 min heating time. The control samples gave an oil yield of 30.40%. It was observed that the oil yield increased after pre-treating the seeds with microwave treatment. This was also observed by Azadmard-Damirchi et al., (2010), Uquiche et al. (2008); Mgudu et al. (2012) on rapeseed, Chilean hazelnut and castor seeds respectively. A high extraction yield can be obtained because the cell membrane is rupture by microwave radiation generating permanent pores thus enabling the oil to move through the permeable cell walls. Uquiche et al., (2008) observed that microwave pretreatment affected both cell walls and the membrane cell (bilayers of lipids and proteins acting as biological barriers).

Microwave Time, s	Moisture content, %	Heating time, min	Heating temp., C	Oil yield, %	Residual oil, %
180	15	5	60	20.16	5.65
240	10	4	80	17.00	5.40
180	15	5	100	18.68	5.35
120	5	5	80	34.45	1.25
120	10	5	100	21.90	4.15
180	5	5	60	35.96	1.26
240	10	6	80	20.30	4.12
240	10	5	60	21.56	4.16
180	10	5	80	22.50	3.88
180	10	4	100	20.65	4.31
180	10	6	100	18.50	5.45
180	5	6	80	35.00	1.66
120	10	5	60	27.30	3.05
240	10	5	100	15.45	6.80
180	10	5	80	22.60	3.65
240	15	5	80	17.40	5.50
180	10	4	60	24.50	3.24
120	10	6	80	23.00	3.95
120	15	5	80	20.02	4.50
240	5	5	80	31.25	2.00
180	5	5	100	32.10	1.95
180	10	5	80	24.00	3.25
180	10	6	60	23.30	3.10
180	10	5	80	22.45	4.04
180	10	5	80	23.50	3.98
180	15	6	80	21.35	3.95
180	5	4	80	36.00	1.05
120	10	4	80	28.50	3.15
180	15	4	80	19.15	6.10

Table 3 Results of process parameters on oil recovery and residual oil content

The oil recovery data were analysed employing multiple regression technique. It shows that the model predicting increased oil yield is significant at 5% level of significance. P values more than 0.05 indicate that the model terms are not significant. The R^2 and the adjusted R^2 values for the studied response variables, were higher than 0.90, hence there is a close agreement between the experimental results and theoretical values predicted by the proposed models.

The predictive best fit regression equation for increased oil yield is:

Oil Yield = +23.03 - 2.45A - 7.62B - 0.31 C -1.99D+0.47AB + 1.65AC - 0.25AD + 0.57BC +0.11BD- $0.28CD - 0.97A^2 + 4.84B^2 - 0.012C^2 - 0.83D^2$

Residual oil yield = +3.76 + 0.66A + 1.82B - 0.085C+0.63D +0.062AB -0.52AC +0.38AD -0.69BC -0.25BD +0.32CD +0.33A² -0.65B² +9.167E-003C² +0.38D² Where, A is microwave; B is moisture content; C is heating time; D is heating temperature.

3.3 Effect of process parameters on oil yield

The effect of moisture content, microwave duration, heating time and heating temperature oil yield is shown in Figure 1. Moisture content had a significant effect on the oil yield. As the moisture content increased, the oil yield decreased. This is because of sliding of seeds along the rotating screw as moisture acts as lubricant in barrel resulting in insufficient friction and less crushing force is applied on the seeds. Same was observed by Akinoso et al., (2006) and Orhevba et al., (2013) on sesame seed and neem seed kernel oil respectively.



Figure 1 Response 3D surface and contour plots for effect of different factors on oil recovery of microwave pre-treated seeds

With the increase in heating temperature the oil recovery was found to be decreased. Kartika et al. (2006) also observed the similar for sunflower seed. While Acheheb et al. (2012) observed that the oil yield increased up to 40° C after this the oil yield decreased. The reduction of oil yield due to increase in temperature would be due to reduction of cake plasticity caused by

water loss at high temperature. Lanoiselle (1994) reported that the temperature and moisture content had a significant combined effect on oil extraction from sunflower seeds.

Microwave duration also affected oil yield. Oil yield deceased with an increase in the microwave time. There was a lower decrease in oil yield from 2 to 3 min but the oil yield decreased more after 3 min of microwave treatment. Kittiphoom and Sutasinee (2015) observed that oil yield increased significantly when microwave pretreatment times was increased from 0 to 60 s however, further increase in microwave pretreatment time from 90-150 s for microwave power did not show any significant improvement. But Azadmard-Damirchi et al., (2010) and Uquiche et al., (2008) reported that increasing the treatment time also had a positive effect on the oil extraction yield for rapeseed and Chilean hazelnut. The residual oil percent was significantly affected by moisture content, heating temperature and microwave treatement.

3.4 Microwave roasting effects on physical and chemical properties of canola oil

3.4.1 Acid value and FFA

The acid value and free fatty acid content of microwave pre-treated canola oil ranged from 3.15 to 5.13 and 1.58 to 2.58 respectively (Table 4). The moisture content and heating temperature affected the acid number of the oil as in Figure 2. With the increase in moisture content and heating temperature the acid value of the oil increased. The microwave treatment also affected the acid value. With the increase in microwave time the acid and FFA value also increased. The similar results were obtained by Yoshida and Kajimoto (1994) and Ozdemir et al., (2006) on sesame oil and tehina oil respectively. The effect of heating time was found non-significant. The free fatty acid content of extracted oil vary the same as acid number of the oil with the optimization factors. The R^2 was 0.7861 while the predicted R^2 was negative which implies that the overall mean is a better predictor of your response than the current model.

seeds							
Microwave, s	Moisture content, %	Heating time, min	Heating temp., °C	Acid mgKOH/g	Number, FFA, %	Peroxide value, meq/kg	
180	15	5	60	4.25	2.13	8	
240	10	4	80	4.19	2.10	9	
180	15	5	100	5.13	2.58	11	
120	5	5	80	3.15	1.58	6	
120	10	5	100	4.60	2.31	8	
180	5	5	60	3.81	1.91	6	
240	10	6	80	4.56	2.29	12	
240	10	5	60	4.10	2.06	9	
180	10	5	80	3.83	1.92	10	
180	10	4	100	4.88	2.45	10	
180	10	6	100	4.20	2.11	10	
180	5	6	80	3.72	1.87	10	
120	10	5	60	3.88	1.95	6	
240	10	5	100	4.78	2.40	12	
180	10	5	80	3.60	1.81	9	
240	15	5	80	4.85	2.43	16	
180	10	4	60	4.05	2.03	10	
120	10	6	80	4.32	2.17	8	
120	15	5	80	3.85	1.93	9	
240	5	5	80	4.25	2.12	8	
180	5	5	100	4.58	2.30	8	
180	10	5	80	3.75	1.88	8	
180	10	6	60	3.52	1.77	7	
180	10	5	80	3.80	1.91	6	
180	10	5	80	3.48	1.75	9	
180	15	6	80	4.39	2.20	10	
180	5	4	80	3.95	1.98	8	
120	10	4	80	4.16	2.09	9	
180	15	4	80	4.46	2.24	16	

Table 4	Results of process param	eters on acid number and po	eroxide value for microwave pr	e-treated
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The multiple regression analysis results the following equations:

Acid Value = +3.69 + 0.23A + 0.29B - 0.082C + 0.38D-0.025AB + 0.052AC - 1.000E - 0.02 AD + 0.040BC+ $0.027BD - 0.038CD + 0.26A^2 + 0.22B^2 + 0.22C^2 + 0.39D^2$ FFA= +1.85+0.11A + 0.15B - 0.040C + 0.19D-1.000E-002AB + 0.027AC - 5.000E-003AD + 0.017BC+ $0.015BD - 0.020CD + 0.13A^2 + 0.11B^2 + 0.11C^2 + 0.20D^2$





Figure 2 Response 3D surface and contour plots for effect of different factors on acid number of oil from microwave pre-treated seeds

3.4.2 Peroxide value

The peroxide value of microwaved canola oil was 6.00 to 16.00 (Table 4). The moisture content had a significant effect on the peroxide value of the oil. Akinoso et al. (2010) also observed the same for sesame oil. Peroxide value depends on the number of factors such as exposure to air, the method of extraction and the type of fatty acids in oil. The high moisture content helps in oxidation of oil which results in rancidity. Heating temperature also had an effect on the peroxide value. Peroxide value increased with the increase in heating temperature. Adeeko and Ajibola (1990) also observed the same for mechanically expressed groundnut oil. This is due to the heating during extraction as heat favours the oxidation of fatty acids mostly polyunsaturated fatty acids.

Microwave treatment also had an effect on the peroxide value. Peroxide value increased with the increase in microwave time this behavior was due to the presence of reactive radicals that might be formed by exposure to microwave. This was also observed by Uquiche et al. (2008) and Oomah et al. (1998) on hazelnut and grapeseed respectively. Figure 3 shows the effect of all the factors. The predicted R^2 is in reasonable agreement with the adjusted R^2 .

The predictive best fit regression equation for peroxide value is

Peroxide Value = +9.24 +1.67A +2.00B -0.42C +1.08D







microwave pre-treated seeds

3.4.3 Density

The density of microwave pre-treated canola seed oil ranged from 833.00 to 911.39 kg/m³ (Table 5). The moisture content of seeds and the heating temperature had a significant effect on the density of oil. As the moisture content increased, there was a decrease in the density of the oil extracted. Increasing the heating temperature decreased the density of oil. Porter and Lammerink (1994) also observed the same. The microwave treatment and heating time had no significant effect on the density of extracted oil as in Figure 4. The predicted R^2 is in reasonable agreement with the adjusted R^2 .

The predictive best fit regression Equation for density is **Density** = +869.87 - 4.56A - 14.35B + 5.53C - 15.31D

Microwave, s	Moisture content, %	Heating time, min	Heating temp., C	Density, kg/m ³	Viscosity, Pa.s
180	15	5	60	845.07	0.0490
240	10	4	80	849.05	0.0432
180	15	5	100	839.49	0.0326
120	5	5	80	887.50	0.0436
120	10	5	100	835.20	0.0387
180	5	5	60	875.00	0.0510
240	10	6	80	847.45	0.0439
240	10	5	60	888.36	0.0521
180	10	5	80	868.72	0.0419
180	10	4	100	833.00	0.0325
180	10	6	100	880.95	0.0322
180	5	6	80	911.39	0.0429
120	10	5	60	885.09	0.0505
240	10	5	100	840.81	0.0350
180	10	5	80	872.09	0.0408
240	15	5	80	863.80	0.0367
180	10	4	60	907.40	0.0440
120	10	6	80	882.50	0.0390
120	15	5	80	869.56	0.0369
240	5	5	80	891.40	0.0435
180	5	5	100	865.41	0.0329
180	10	5	80	879.12	0.0388
180	10	6	60	877.66	0.0465
180	10	5	80	849.42	0.0420
180	10	5	80	886.79	0.0377
180	15	6	80	875.00	0.0362
180	5	4	80	888.88	0.0440
120	10	4	80	875.79	0.0377
180	15	4	80	854.44	0.0362







Figure 4 Response surface and contour plots for effect of different factors on density of oil from microwave pre-treated seeds

3.4.4 Viscosity

The viscosity of the microwave pre-treated canola seed oil was in a range of 0.0322 to 0.0521 pa s (Table 5). The heating temperature and the moisture content had a significant effect on the viscosity of the oil. The viscosity of the oil decreased with the increase in temperature. This was also reported by Diamante and Lan (2014) for some vegetable oils including canola. As observed by Diamante and Lan (2014), the canola oil viscosity decreased from 0.0462 Pas to 0.0108 Pas as temperature increased from 30° C to 90° C. The moisture content also had an inverse relationship with viscosity. This was also observed by Adejumo et al. (2013). The microwave treatment and heating duration had no significant effect on the oil viscosity as is shown in Figure 5. The predicted R^2 is in reasonable agreement with the adjusted R^2 .

The predictive best fit regression equation for peroxide value is:

Viscosity = +0.041 +6.667E-004A -2.525E-003 B +2.583E-004C -7.433E-003D



Figure 5 Response 3D surface and contour plots for effect of different factors on viscosity of oil from microwave pre-treated seeds

4. Conclusion

In this study, the effect of microwave (MW) pretreatment on extraction oil yield and oil quality from Canola (Variety GSC-6) was investigated. The

conclusion reached was that microwave pre-treatment can be used as a desirable alternative to conventional oil extraction techniques and could be applied to canola oil extraction before pressing to improve oil yield. Maximum oil yield of 36% was obtained. MW pre-treatment helps in the rupture of the cell wall making cell permeable and enabling the oil to move through the permeable cell walls. There was a little effect of microwaves on the oil quality of canola. The study found that optimum condition for mechanical extraction of canola (GSC-6) using is 5% moisture content, 120 s microwave time, 6 min heating time and 60 °C heating temperature with predicted values 87.90%, oil recovery and 1.72% residual oil in cake, acid number 3.72, peroxide value 4.10, density 909.22 kg/m², viscosity 0.0501 Pa.s. The effect of all the parameters except heating time was found to be significant on oil yield. time, moisture content and heating Microwave temperature affected the acid value and the peroxide value of the oil. Heating temperature affected the viscosity of the oil while other parameters had no effect.

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