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Effect of Ohmic Heating Pre-treatment on Millable Oil Extraction and Physicochemical Properties of Mustard (*Brassica juncea*) Oil

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ABSTRACT: The pre-treatment of seeds plays a crucial role in enhancing oil extraction efficiency. Present study aimed to evaluate the effect of ohmic heating pre-treatment on the extraction of millable oil and physicochemical properties of the oil extracted from mustard seeds. The oil yield, peroxide value, acid value, free fatty acid (FFA) content, colour, refractive index, density and viscosity of the extracted oil were evaluated under varying electric field strengths (EFS) and exposure times. The results showed that ohmic heating pre-treatment significantly increased the oil yield compared to the untreated sample, with the highest recovery achieved at an EFS of 24.5 V/cm and an exposure time of 600 s. However, higher EFS levels and longer exposure times led to undesirable effects on the oil quality, as indicated by increased peroxide value, acid value and FFA content. The colour of the oil varied with EFS and exposure time, showing a decrease with higher EFS and an increase with longer exposure times. The refractive index, density, and viscosity of the oil remained relatively unchanged. These findings suggest the potential of ohmic heating as a pre-treatment strategy for enhancing oil extraction efficiency from mustard seeds, with minimal changes in terms of oil quality.

Keywords: Ohmic heating, mustard seeds, oil extraction, physicochemical properties.

INTRODUCTION

Mustard seeds are an essential agricultural commodity known for their rich oil content, making them a valuable source for oil extraction in various industries (Grygier, 2022). The extraction process involves the use of several procedures targeted at increasing oil yield and enhancing product quality. Among the various these methods, pre-treatment using ohmic heating has shown potential for improving oil extraction efficiency while keeping the physicochemical characteristics of mustard seed oil within the acceptable range.

The primary factor in the oil extraction process is the availability of millable oil from mustard seeds. Various pre-treatment methods have been explored to increase the availability of oil within the seeds, thereby improving overall oil extraction efficiency. These methods use different principles to facilitate the release of oil from the seed matrix. Microwave heating employs electromagnetic waves to cause fast molecular vibration within the mustard seeds, resulting in improved oil extraction (Koubaa *et al.*, 2016). Infrared heating, on the other hand, utilises infrared radiation to penetrate the seeds, promoting efficient heat transfer and facilitating oil release (Aboud *et al.*, 2019). Ohmic heating involves the passage of electric current through the seeds, leading to controlled heating and subsequent oil release (Kumari *et al.*, 2016).

The physicochemical properties of the extracted oil play a crucial role in determining its suitability for consumption and other applications. Parameters such as acid value, peroxide value, free fatty acid (FFA) value, viscosity, density, refractive index, and colour are commonly used to assess the quality and stability of vegetable oils. The acid value measures the free fatty acid content, indicating the degree of hydrolysis and rancidity. The peroxide value reflects the extent of oxidation and the potential for the formation of rancid flavours and odours. The FFA value is an important parameter indicating the purity and potential for rancidity of oil (Ren *et al.*, 2018). Viscosity determines the flow characteristics and ease of handling the oil. Density provides information on the mass of the oil per unit volume, affecting its storage and transportation. Refractive index is a measure of the optical properties of the oil, while colour is a visually perceptible characteristic influencing consumer acceptability and product application (Moghimi *et al.*, 2018). The effects of pre-treatment techniques on these physiochemical characteristics can be seen as indicators of the quality, shelf life and market value of the oil.

Limited research has been conducted on the effects of ohmic heating on mustard seed oil extraction and the resulting physiochemical properties. However, existing studies on other oilseeds have demonstrated the potential benefits of this pre-treatment. For instance, ohmic heating has been investigated for its potential to increase oil recovery and improve the nutritional and sensory properties of extracted oils (Kumari *et al.*, 2016).

This study aims to investigate the effect of ohmic heating on the availability of millable oil from mustard seeds and the physiochemical properties of extracted oil, including peroxide value, acid value, FFA content, colour, refractive index, viscosity and density.

MATERIAL AND METHODS

A. Materials

The mustard (*Brassica juncea*) seeds (*var*. RH-0749) were collected from the National Seed Corporation in India. The selected seeds had a moisture content (w.b.) of 7% (± 0.02) and an average diameter of 1.6 ± 0.4 mm. The selected seed samples were subsequently subjected to ohmic heating pre-treatment as per the experimental conditions.

Pre-treatment of mustard seeds

Mustard seeds were used in the study in batches of 250 g for pre-treatment. In order to achieve homogeneous heating, the moisture content of raw mustard seeds (moisture content (w.b.) 7%) was increased by water-soaking in a beaker, which ensured the consistent flow of electric current across the sample. Preliminary tests were conducted at moisture content levels of 20, 25, 30 and 35% (w.b.) by adding 40, 60, 82.14 and 107.7g of water per 250 g of mustard seed. After tests, the mustard seeds with a moisture content level of 35% were the most suitable for consistent flow of electric current to achieve the desired endpoint temperature of 90 ºC. The sample then subjected to electric filed strengths (EFS) of (22, 24.5 and 27 V/cm) at different holding time (HT) (300, 600 and 900 s).

B. Mustard seeds oil extraction

The pre-treated mustard seeds samples were pressed in lab scale cold press expeller (Make: SH – 400, Shreeja Products, India). The crude oil obtained after pressing and seed disintegration was filtered using a sieve to remove larger impurities, followed by a secondary filtration with a muslin cloth to eliminate small suspended particles. The filtered crude oil was then collected in 250 mL plastic bottles and stored under refrigerated conditions.

C. Physicochemical analysis

Peroxide value. Peroxide value (PV) is a measurement of the level of peroxides present in the oil, which is one of the measures of level of its rancidity. The peroxide value of mustard oil determined using the AOCS Official Method Cd 8-53 (AOAC, 2000). Accordingly, a crude oil sample of 5 ± 0.5g was taken and placed in a 250 mL conical flask. The test sample was protected from light and air exposure during the entire process, to prevent oxidation. Further 30 mL of an acetic acid-chloroform solvent in a 3:2 ratio was added to the sample. Followed by the addition of 0.5 mL of freshly prepared saturated potassium iodide solution and the flask was shaken for 1 min to mix the solution. About 30 mL of water was added further and solution allowed for titration with 0.1 N sodium thiosulfate. The titration was continued until the yellow iodine tint had completely disappeared. The volume of the sodium thiosulfate solution used in the titration was recorded and used to calculate the peroxide value. The peroxide value was calculated using the formula:

$$\text{PV (meq/kg)} = \frac{(A - B) \times N \times 1000}{W} \quad (1)$$

Where, A = mL of sodium thiosulfate used for titration of the sample.

B = mL of sodium thiosulfate used for titration of blank.

N = Normality of the sodium thiosulfate solution.

W = mass of the oil sample in g.

Acid value. The acid value of mustard oil can be determined using the AOCS Official Method Cd 3d-63, with slight modification as per Kardash & Tur'yan, (2005) which is based on the neutralisation of the free fatty acids present in the oil with a standard solution of potassium hydroxide (KOH). During estimation of AV a known weight of mustard oil (10 ± 0.02 g) was dissolved in 50 mL of isopropyl alcohol in a 250 mL conical flask and few drops of phenolphthalein indicator was added into the solution. Then mixture was then heated at 75 ± 5°C of temperature for about 15 min. The mixture is then titrated with a standardised solution of potassium hydroxide (KOH, 0.1N) until the endpoint was reached. The endpoint was indicated by a change in the colour of phenolphthalein indicator, from colourless to pink. The acid value was calculated by the following expression:

$$\text{Acid value (mg KOH/g)} = \frac{56.11 \times V \times N}{W} \quad (2)$$

Where, V = Standard Potassium hydroxide volume in mL

N = Normality of the Potassium hydroxide solution

W = Sample weight in g

The test was conducted at room temperature (about 25°C) and the endpoint was reached within 15 min. The accuracy of the test was assured by using high-quality samples that have been properly stored and handled.

FFA. The calculation of free fatty acid (FFA) content in edible oils is typically represent the percentage of the major fatty acid present in it i.e., oleic acid. Accordingly, the FFA present in the oil samples was determined by using standard methodology given by FSSAI, (2021). Therefore, FFA in terms of oleic acid content present in the oil sample was estimated as follows:

$$\text{\% FFA as oleic acid} = \frac{AV}{1.99} \quad (3)$$

Refractive Index. The refractive index of mustard oil was determined using an Abbe refractometer (AR-12, Weswox Scientific Equipment Pvt. Ltd., Haryana, India) as per the standard procedure described in AOAC 921.08 (2000). During the test the sample of oil was maintained at temperature around 25 °C and it was ensured to free from bubbles and particles. The prism of the refractometer was cleaned with a lint-free cloth and a suitable solvent (ethyl alcohol) to remove any dirt or residue. The instrument was then calibrated using a reference liquid with a known refractive index like solution of sodium was chloride (NaCl). Day light was used as a light source which would reflect prism with the help of reflector. Measurement was conducted by opening the double prism with the help of the screw head, followed by placing a droplet of the oil sample on the prism followed by spreading it evenly, and the recording of the reading from dial.

Viscosity. The Sine-wave Viscometer (SV-10, A&D vibro viscometer, A&D Limited, Japan) with an accuracy of 1% was used for the measurement of the viscosity of oil samples. During the test, the sensor plates of the viscometer were first cleaned with a wet tissue paper to remove any traces of the previous sample and then dried using a dry tissue paper to remove moisture. The viscometer was checked for accuracy using distilled water at 20°C before measuring the viscosity of the samples. Around 40 mL of the oil sample was placed in a polycarbonate auxiliary sample cup (AX-SV-33). The cup was securely fixed on the stage to prevent any vibrations. The test was performed when the sensor plate had been submerged in the sample. The viscosity and temperature of the sample were presented separately by the device, while the viscosity value reported separately (Echalier *et al.*, 2017).

Density. The density of the mustard oil was measured by weighing a clean and dry relative density (RD) bottle using a calibrated balance as mentioned by Vani *et al*. (2019). Hence during the test, first the mass of the empty bottle was recorded. The RD bottle was then filled with mustard oil up to the specified volume mark, avoiding air bubbles to maintain the accuracy of the measurement. The bottle and the oil are then weighed and the mass was recorded. The temperature of the mustard oil and the RD bottle was maintained the same throughout the measurement. Accordingly, the density of the mustard oil was calculated using the formula:

$$\rho = \frac{M}{V} \tag{4}$$

Where, ρ = Density in g/mL.
M = Mass of mustard oil in g.
V = Volume of RD bottle in mL.

Colour. Photometric colour index (PCI) method was used to assess the colour of the oil. The colour value (C) of the oil was determined using a spectrophotometer (Shimadzu UV-1800 UV/Visible, 115 VAC, Columbia, MD, USA). Prior to analysis, the spectrophotometer was calibrated using distilled water as a blank. Oil samples were collected in 4 mL disposable cuvettes, and any excess oil was wiped from the cuvette exterior using tissue paper. The absorbance (A) of each oil sample was measured at four wavelengths: 427, 453, 482, and 670 nm. The PCI of the oil (C) was then calculated using the formula described by Seth *et al*. (2010).

$$C = 1.29A_{427} + 69.7A_{453} + 41.2A_{482} - 56.4A_{670} \tag{5}$$

D. Statistical analysis

The statistical analysis was performed using IBM SPSS Statistics v20 software. A full factorial design analysis was conducted through ANOVA. Tukey's post hoc test was used to identify significant differences between treatment groups. It compared all the possible pairs of treatment means and determined if they were significantly different from each other.

RESULTS AND DISCUSSION

A. Effect of ohmic heating on extraction of millable oil from mustard seed

Pre-treatment is a crucial step to get a desired extractability of oil from seeds as it altering physical and chemical properties of the oil present in seeds and make them more amenable to extraction. The effect of ohmic heating used as pre-treatment, with specific EFS and exposure time, on the millable oil recovery from mustard seeds reveled that the oil yield varied from 32.28% to 33.18%, (Fig. 1). The untreated sample exhibited an oil yield of 26.41%. A noticeable increase in the millable oil recovery at EFS of 22 V/cm was observed during the experimentation. In contrast, no significant increase in oil recovery was observed at 24.5 V/cm and 27 V/cm. However, concerning exposure time, the recovery was found to show no significant increase at 300 s, whereas an increase in oil recovery was observed at exposure times of 600 s and 900 s, as depicted in Table 2. Mainly, for the ohmic heating highest oil recovery of 33.18% was obtained under the conditions of the EFS (24.5 V/cm) and exposure time (600 s).

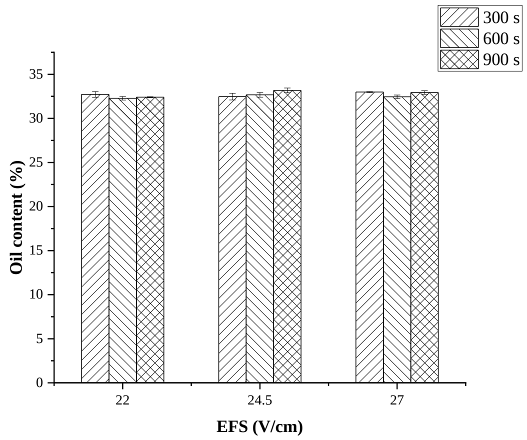

Fig. 1. Oil content of OH treated mustard seeds observed at varying EFS and exposure time.

The ANOVA shown in Table 1 represent the significance of the effect of variables and their combinations and corroborated the results obtained. The statistical analysis confirmed the regression models represent the effect on the yield of millable oil content, with F value of 4.75 ($p<0.01$) and an R^2 value of 0.68. Additionally, the linear and interaction effects of the two factors, EFS and exposure time on the millable oil recovered from ohmic heating treated mustard seeds were found to be significant ($p<0.05$) (Table 2). The similar outcome was observed for sesame seeds (Kumari *et al.*, 2016), mustard seeds (Gajanan *et al.*, 2017) and rice bran (Lakkakula *et al.*, 2004).

The application of an electric current during the ohmic heating pre-treatment may enhance the breakdown of cell membranes and promote the release of oil from the oil globules present in the mustard seeds. The combination of electroporation, which allows for the creation of temporary pores in the cell membranes, and thermal softening, which reduces the viscosity of the oil, can contribute to increase in mass transfer rate and hence improved oil extraction efficiency (Praporscic *et al.*, 2006).

Table 1: ANOVA (F-values) for oil extracted and their physicochemical parameters of OH treated mustard seeds.

Source	Oil content	PV	AV	FFA	Colour
Model	4.75	142.108	848.534	848.53	28.61
EFS	4.96	176.746	294.532	294.53	61.66
HT	5.64	304.643	2837.795	2837.80	33.65
EFS × HT	4.19	43.52	130.903	130.90	9.57

Table 2: Mean value of depending variables affected by EFS and HT of OH pre-treated mustard seeds.

Parameters	Oil content (%)	PV (meq/kg)	AV (mg KOH/g)	FFA	Colour
EFS					
22.0 V/cm	32.47±0.28[a]	0.98±0.04[a]	1.82±0.07[a]	0.91±0.03[a]	422.97±1.35[a]
24.5 V/cm	32.77±0.41[b]	0.98±0.01[ab]	1.86±0.07[b]	0.93±0.04[b]	421.00±1.91[b]
27.0 V/cm	32.79±0.30[bc]	1.03±0.05[c]	1.87±0.08[c]	0.94±0.04[c]	418.57±2.34[c]
HT					
300 s	32.73±0.34[a]	0.96±0.01[a]	1.77±0.03[a]	0.89±0.02[a]	419.11±2.62[a]
600 s	32.47±0.26[ab]	1.00±0.02[b]	1.84±0.02[b]	0.92±0.01[b]	421.09±2.93[b]
900 s	32.84±0.38[ac]	1.03±0.04[c]	1.94±0.04[c]	0.97±0.02[c]	422.34±0.69[c]

Matched alphabetic superscripts in subheadings indicate statistically similar numerical values.

B. Effects of ohmic heating on peroxide value of milled oil

The peroxide value indicates the hydroperoxides content, which are primary oxidation components in the oil. The effect of ohmic heating on the peroxide value of oil extracted from mustard seeds resulted in the variation of peroxide value from 0.94 to 1.08 meq/kg, depending on the experimental conditions (Fig. 2). The peroxide value of the untreated sample was 0.91 meq/kg. The results indicate no significant increase in the peroxide value when the EFS level was increased from 22 V/cm to 24.5 V/cm. However, at 27 V/cm, there was an increase in peroxide value. Additionally, Table 2 shows that there was a significant increase in peroxide value with increase in exposure time.

The ANOVA shown in Table 1 corroborated the results of peroxide value. The regression model's shows best fit for prediction of the peroxide value of mustard oil with F value of 142.108 ($p<0.01$) and an R^2 value of 0.98. Furthermore, the study found that the linear and interaction effects of the two factors, EFS and exposure time, on the peroxide value from OH-treated mustard seeds were significant ($p<0.01$) (Table 2). This suggests that higher EFS levels during ohmic heating pre-treatment may promote oxidation reactions and the formation of peroxides in the oil. These findings are in line with previous studies that have reported an increase in peroxide value with higher electric field strength during ohmic heating (Gajanan *et al.*, 2017; Karunanithi *et al.*, 2019).

C. Effects of ohmic heating on acid value of milled oil

The acid value is an important parameter for evaluating the quality and stability of edible oils. The results indicated that the acid value varied from 1.74 to 1.98 mg KOH/g under different experimental conditions (Fig. 3). The acid value of the untreated sample was 1.73 mg KOH/g.

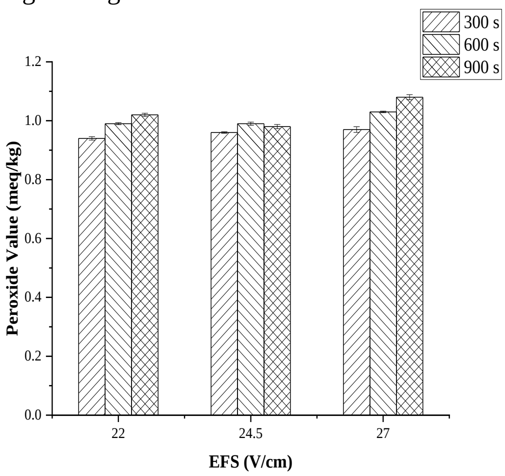

Fig. 2. Peroxide value of oil extracted from OH treated mustard seeds observed at varying EFS and exposure time.

The study found a significant increase in the acid value with an increase in EFS and exposure time (Table 2). The regression analysis yielded an F value of 848.53 ($p<0.01$) and an R^2 value of 0.99 and hence found to be bet fit equation for prediction of acid value of the samples (Table 1). Moreover, the study indicated that the linear and interaction effects of the two factors, EFS and exposure time, were significant ($p<0.01$) in relation to the acid value of oil extracted from OH-treated mustard seeds, as shown in Table 2. The increased EFS and longer exposure times lead to higher temperatures, which can accelerate the hydrolysis of triglycerides into free fatty acids, contributing to the increased acid value of the extracted oil. A similar observation was reported for sesame seeds (Kumari *et al.*, 2016).

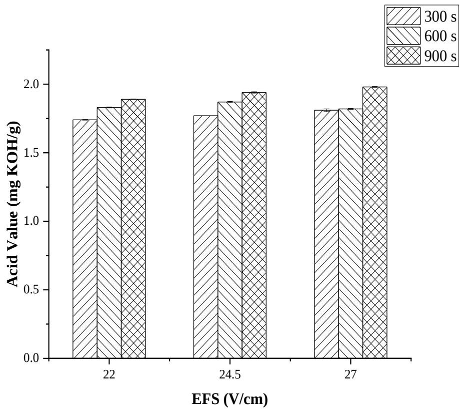

Fig. 3. Acid value of oil extracted from OH treated mustard seeds observed at varying EFS and exposure time.

D. Effects of ohmic heating on free fatty acid (FFA) content

The effect of ohmic heating on the FFA content of oil extracted from mustard seeds was investigated by altering the EFS and exposure time. The results indicated that the FFA content varied from 0.87 to 1.00% (Fig. 4). The FFA content of the untreated sample was 0.87%. The study found a significant increase in the FFA content with an increase in EFS and exposure time (Table 2). The ANOVA analysis also supported the results, confirming the considerable effect of the regression model could significantly represent the change in the FFA content of mustard oil. The statistical analysis yielded an F value of 848.53 ($p<0.01$) and an R^2 value of 0.99 (Table 1). Moreover, the study indicated that the linear and interaction effects of the two factors, EFS and exposure time, were significant ($p<0.01$) in relation to the FFA content of oil extracted from treated mustard seeds, as shown in Table 2. The hydrolysis of oil, which may be due to water and lipase enzyme activity, might have led to increased FFA value with temperature (Budijanto *et al.,* 2008; Gajanan *et al.,* 2017).

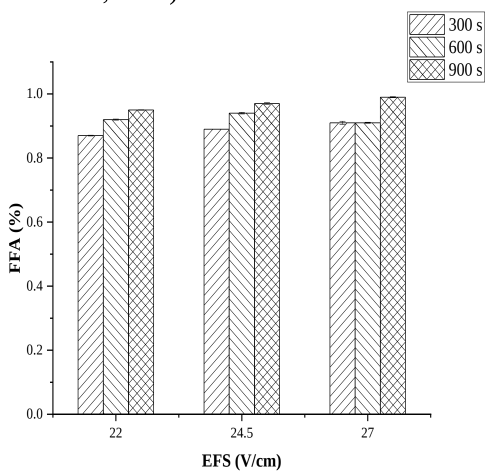

Fig. 4. FFA content of oil extracted from OH treated mustard seeds observed at varying EFS and exposure time.

E. Effects of ohmic heating on colour of milled oil

The results of the colour value of oil extracted from mustard seeds indicated that the colour value ranged from 416.74 to 424.24 at various experimental conditions (Fig. 5). The colour value of the untreated sample was 404.35. The study found a significant decrease in the colour values with an increase in EFS and a significant increase in the colour values were observed with an increase in exposure time (Table 2).

The regression model was found to be represent the change in colour values with an F value of 28.61 ($p<0.01$) and an R^2 value of 0.93 (Table 1). Finally, in the study ANOVA of the experimental data shown that the linear and interaction effects of EFS and exposure time were significant factors in relation to the colour value of oil extracted from OH-treated mustard seeds, as shown in Table 2. Karunanithi *et al.* (2019) also reported variation observed after the ohmic heating treatment of tomato seeds.

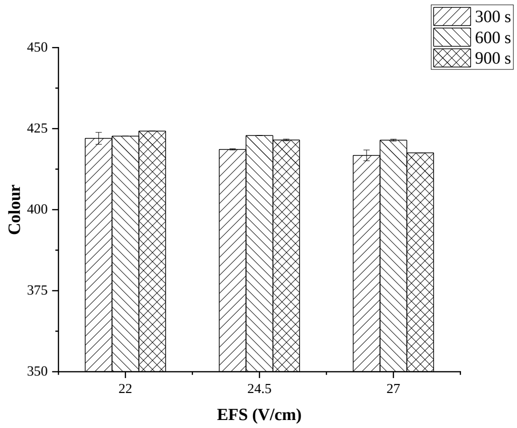

Fig. 5. Colour of oil extracted from OH treated mustard seeds observed at varying EFS and exposure time.

F. Effects of ohmic heating on refractive index, density and viscosity of milled oil

The refractive index value for untreated mustard oil was 1.471, while the ohmic heating treated oil, it was found in the range of 1.473 to 1.475. The findings show that the refractive index of mustard oil was not significantly affected by the pre-treatment methods, showing no definite pattern of increase or decrease with the increase in the treatment levels of any pre-treatment methods. Therefore, the study concluded that the effect of pre-treatment methods on the refractive index of oil was insignificant ($p<0.05$). Similar observation is also reported for microwave pre-treated pumpkin seeds (Jiao *et al.,* 2014).

The viscosity of untreated mustard oil was found to be 24.6 mPa.s, while the viscosity of ohmic heating treated oil ranged from 23.8 to 25.0 mPa.s. Similar to the refractive index, no regular pattern was observed in the viscosity of oil with a change in pre-treatment levels. Therefore, the study found no statistically significant ($p>0.05$) difference in the viscosity of the oil before and after pre-treatment.

The density of pre-treated mustard oil was also measured; untreated mustard oil was found to be 9010 kg/m^3, while the density of ohmic heating treated oil ranged from 9010 to 9030 kg/m^3. The results of the study indicate that the viscosity of mustard oil treated with different pre-treatment methods varied within a narrow range. Therefore, the study found no statistically significant (p>0.05) difference in the density of the oil before and after pre-treatment.

When comparing traditional extraction methods with advanced technologies such as ohmic heating, it is observed that the physicochemical properties of oil, such as refractive index, density and viscosity remain unchanged (Gorji *et al.*, 2015; Karunanithi *et al.*, 2019).

CONCLUSIONS

This study investigated the effect of ohmic heating pre-treatment on the extraction of millable oil from mustard seeds and the physicochemical properties of extracted oil. The oil yield was significantly improved compared to the untreated sample, with the highest oil recovery (33.18%) obtained at an electric field strength (EFS) of 24.5 V/cm and an exposure time of 600 s. This increase in oil recovery can be attributed to the breakdown of cell membranes and the promotion of oil release through electroporation and thermal softening.

However, the increase in EFS and longer exposure times during ohmic heating led to undesirable effects on the quality of the extracted oil. The peroxide value, which indicates the presence of hydroperoxides and primary oxidation components, increased significantly with higher EFS levels and exposure times. This suggests that higher EFS levels promote oxidation reactions and the formation of peroxides in the oil. Similarly, the acid value and free fatty acid (FFA) content of the oil increased with increased EFS and exposure time, indicating hydrolysis of triglycerides into free fatty acids.

Regarding the colour of the extracted oil, an increase in EFS led to a significant decrease in colour value, while longer exposure times resulted in an increase. The refractive index, density, and viscosity of the oil were not significantly affected by the ohmic heating pre-treatment.

Overall, ohmic heating pre-treatment improved the oil recovery from mustard seeds, but it also had some detrimental effects on the quality of the extracted oil, as indicated by increased peroxide value, acid value, FFA content, and variable colour changes.

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Conflict of Interest. None.

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