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ORIGINAL PAPER

Comparison of Ethyl Acetate with Hexane for Oil Extraction from Various Oilseeds

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Abstract The aim of the current research was to determine a less hazardous, cheaper and less toxic alternative solvent for hexane for extraction of oil from different oilseeds showing equivalent oil yield and oil quality. A full factorial design with three levels of extraction temperature (80, 100 and 120 °C) and three levels of extraction time (40, 65 and 90 min) with constant solvent to seed ratio value of 4:1 was used to extract the oil. Maximum oil was recovered from canola followed by flax, mustard and camelina. The oil content of canola was found in range of 21.08–36.44, and 25.12–40.38 % for hexane and ethyl acetate, respectively. The heating values of oil extracted from all oilseeds using hexane and ethyl acetate were found in the range of 38.04–39.98 and 37.98–39.37 MJ/kg, respectively. Least viscosity was found for flax seed using hexane followed by camelina, canola and mustard as compared to ethyl acetate. Viscosity of flax oil ranged from 27.23–37.19, and 31.16–55.52 cP for hexane and ethyl acetate solvents, respectively. Considering human safety, less environmental impact, comparable oil yield and quality parameters, ethyl acetate can be a promising alternative to hexane.

Keywords Hexane · Ethyl acetate · Canola · Flax · Mustard · Heating value · Viscosity

Abbreviations

ASE	Accelerated solvent extractor
Cn	Canola
Cm	Camelina
EA	Ethyl acetate
Fx	Flax
H	Hexane
MC	Moisture content
Mt	Mustard

Introduction

Oilseeds are an important economical factor in world trade of agricultural products. The oil content of seed is a key interest to the oil industry because the monetary assessment in the trade of oilseeds is based on this value as now a days, oil from oilseeds is being upgraded to biodiesel or jet fuel to overcome the future crisis of fossil fuels. Solvent extraction for oilseeds is an efficient approach and currently, hexane is being used as a solvent obtained from petrochemical sources. Hexane can be emitted to the environment during extraction and recovery and can react with other pollutants to produce ozone and photochemical oxidants [1]. Safety, environmental and health concerns which was prompted by the 1990 Amendments to the Clean Air Act of USA and its testing as a hazardous air pollutant, have stimulated interest in alternative solvents [2].

As an alternative to organic solvent extraction, the extraction of oil from oilseeds and other oil-containing materials with aqueous solutions has been investigated [1]. Low selectivity of aqueous solutions for lipids, results in simultaneous extraction of proteins, carbohydrates and other compounds from materials into oil. Therefore, the most feasible alternative to hexane extraction seems to be the other organic solvents known to be environmentally safer. Plenty of research works have been done to replace hexane with other hydrocarbons, or

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alcohols as solvents for oil extraction. *n*-heptanes, and isohexane hydrocarbon solvents were recommended as potential substitutes for hexane to extract oil from cottonseed [3] and soybean [2]. Among the alcohols, isopropanol and ethanol are the most promising solvents for the oil extraction from sunflower seed [4], soybean [5], rice bran [6] and sesame seed [7]. Petroleum benzene [8] and petroleum ether [9] were also used to extract the oil from rapeseed, sunflower, soybean, and flaxseed, respectively. Some green solvents like limonene, and pinene were also tried to replace the hexane for the extraction of rice bran oil [10], olive [11] and microalgae [12].

Keeping in view the above facts, a preliminary study (unpublished) was conducted to extract the oil from canola seed (*Brassica napus*) in an accelerated solvent extractor (ASE) using different solvents. Solvents were categorized as hydrocarbon (*n*-hexane), alcohol (ethanol and butanol-1), green (pinene), and ester (ethyl acetate). A full factorial design was used to determine the highest oil yield for each solvent as a function of temperature, time, and solvent to seed ratio (R). All three variables were varied at two levels of 80 and 120 °C, 40 and 90 min, and 3:1 and 5:1, respectively. It was observed that with increase in temperature and extraction time, oil yield increased. There was no significant increase in oil yield with increases in the solvent to seed ratio. For canola seed, oil extraction using hexane and ethyl acetate at 120 °C temperature, 90 min extraction time and a 5:1 solvent to seed ratio resulted in the maximum oil yield as 43.08 and 41.39 %, respectively. Pinene and butanol have high viscosities and, because of that, even at high temperature (120 °C), oil recoveries were less as compared to hexane and ethyl acetate. Ethanol solvent resulted in the least oil yield (20.65 %). Therefore, ethyl acetate was selected for comparison with hexane for extraction of oil from different oil seeds in further study.

The aim of this study was to investigate the effect of extraction parameters on the yield of extracted oil for *n*-hexane and ethyl acetate solvents. On the basis of preliminary study (unpublished), a full factorial design was used as a function of the following variables: extraction temperature and time with a constant solvent to seed ratio. Both the solvents were compared for oil yields and qualities of different oilseeds.

Materials and Methods

Canola (*Brassica napus*), camelina *Camelina sativa* (BSX-WG-1), flax (York) and mustard (Kodiak) were procured from Seed house, South Dakota State University. All seeds were ground in a Wiley mill to pass a 2-mm screen. Moisture contents of all seeds were found in the range of 7.5–9.0 % which was suitable (<10 %) for solvent extraction. All seeds were packed in airtight poly bags and stored at 4 °C for further extraction in ASE.

Different solvents, i.e. *n*-hexane, ethyl acetate, ethanol, butanol-1 and pinene were procured from Fisher Scientific. All solvents were stored in safety cabinet at room temperature.

Solvent Extraction

An accelerated Solvent Extractor (ASE 350, Dionex) was used to extract the oil from canola (Cn), camelina (Cm), flax (Fx) and mustard (Mt) oilseeds using hexane and ethyl acetate. Oils were extracted at three different temperatures, i.e. 80, 100 and 120 °C for 40, 65 and 90 min of extraction time. The solvent to seed ratio was kept constant as 4:1 from preliminary experiments. The system pressure was controlled at 1500 psi throughout the experiments.

Soxhlet Extraction

Briefly, 2 g of ground oilseeds were extracted in a Soxhlet apparatus with 30 mL of boiling *n*-hexane for 8 h. The mixture of oil and hexane was collected, concentrated under vacuum, and dried for 5 min at 105 °C. The extraction yield was determined using the official recommendation [13].

Moisture Content

Moisture content (MC) of ground oilseeds was determined by air oven standard methods recommended by the AOAC [14]. Initially 5 g of sample in triplicate was dried in hot air oven at 130–133 °C for 2 h. After drying, the dried sample was again weighed. The following formula was used for calculating the MC.

$$\text{MC (wb, \%)} = \frac{W_\mathrm{i} - W_\mathrm{f}}{W_\mathrm{i}} \times 100$$

W_i = initial weight of sample (5 g), W_f = weight of sample after drying, g

Oil Characteristics

Unsaponifiable Matter

Unsaponifiable matter of the oils was determined according to AOCS Recommended Practice Ca 6a-40 [15] and was expressed as a percentage (w/w) of oil.

Density

Density measurements were carried out using a pycnometer at a temperature of 25 °C (±0.1 °C). The pycnometer of capacity 25 mL was calibrated with water.

Viscosity

Viscosity measurement was carried out using a visco-analyzer rheometer equipped with commercial computer software (RheoExplorer 39E). The experiments were conducted using a concentric cylinder geometry consisting of a rotational inner cylinder (bob) with an outer radius of 25 and 44.5 mm immersed height. The bob rotated in an outer stationary cup having 26 mm inner radius and thus giving a 1-mm gap for fluid to be sheared. Measurements were taken for 15-ml samples at 25 °C (±0.1 °C) controlled temperature. The viscometer operated at forty fixed shear rates from 0 to 200 s^{-1}.

Heating Value

An oxygen bomb calorimeter (IKA C2000) was used to determine the heating value of the oil samples. A known quantity of oil was placed in the cup inside the bomb filled with oxygen for combustion. The instrument gave the direct heating value calculated from temperature increased in the water surrounding the jacket after combustion of a known amount of sample.

FFA Analysis

First, 20 mg oil was mixed with 2 ml hexane and 2 ml BF_3 methanol in a 20-ml conical flask. The mixture was then heated in a water bath at 60 °C for 1 h followed by cooling down to room temp. The mixture was topped up with 2 ml distilled water then was shaken for 10 min in a shaker to get two different layers. Sometimes, it was necessary to centrifuge the mixture at 2500 rpm for 10 min to get layers. The upper layer was decanted into vials and a small amount of sodium sulfate was added to the vial to absorb the water from the mixture. After 10 min, the mixture was filtered through a 2-μm syringe filter to remove the salt impurities. After diluting the mixture with hexane in a 1:1 ratio, the derivatized sample was run in GC–MS (Agilent GC- 7890A and MSD-5975C) to identify the free fatty acids present in the oil sample. The operating conditions were injection port temperature, 250 °C; interface temperature, 250 °C; column oven temperature, 75 °C for 2 min, ramped at 15 °C min^{-1} to 250 °C with a 2-min hold; helium carrier gas (flow rate of 1 mL min^{-1} at 75 °C). The injector was operated in the splitless mode for 1 min after injection of the sample and a capillary column (30 m × 0.25 mm × 0.25 mm DB-5MS) was used.

Statistical Analysis

Full factorial design was used for experimental plan and results were compared by analysis of variance (ANOVA) using SPSS (16.0) statistical software. All data were reported as means ± standard deviations of replicates. Tukey's tests were used to compare the significant differences of the mean values with the family error rate held at 0.05.

Results and Discussion

Effect of Extraction Time and Temperature on Oil Yield

ANOVA showed that the effects of extraction time and temperature were significant ($p < 0.05$) for all experimental oilseeds for both the solvents (data not shown). Oil yield for different oilseeds increased with increasing extraction time and temperature. More extraction time allows more fat to diffuse into the extraction solvent. As the temperature increased, the viscosity of the solvent was reduced, thereby increasing its penetration into the sample matrix and solubilize the analyte (fat). The addition of thermal energy also assists in breaking analyte matrix bonds and encourages analyte diffusion from the matrix surface [16].

The oil yield of canola for hexane and ethyl acetate exhibited a range of 21.08–36.44, and 25.12–40.38 %, respectively (Table 1). These values for hexane were in accordance with the findings reported by Latif *et al.* [17]. Up to 100 °C extraction temperature, the hexane extracted oil yield did not show any significant ($p > 0.05$) difference when extraction time increased from 65 min to 90 min, while ethyl acetate extracted oil yield increased significantly ($p < 0.05$). At high temperature of 120 °C, only ethyl acetate extracted oil yield showed the significant ($p < 0.05$) increase with increase in extraction time from 65 to 90 min. It may be due to higher boiling point of ethyl acetate compared to hexane. Stabilized viscosity of hexane at 40 min and 120 °C temperature resulted in optimum diffusion of oil into solvent. At 40 min of extraction time, increase in temperature from 80 to 120 °C had no significant ($p > 0.05$) effect on oil yield using ethyl acetate, whereas, hexane extracted oil yield significantly ($p < 0.05$) increased from 80 to 100 °C. Increasing the temperature from 100 to 120 °C at 65 min of extraction time exhibited a significant ($p < 0.05$) increase in oil yield only for ethyl acetate extraction. For both the solvents, oil yield did not show any significant ($p > 0.05$) increase when extraction temperature increased from 100 to 120 °C at 90 min of extraction time. For canola seed, oil extraction using hexane and ethyl acetate at 120 °C temperature and 90 min extraction time resulted in the maximum oil yield as 36.44 and 40.38 %, respectively (Table 1).

Camelina oil was extracted by hexane and ethyl acetate and the oil content ranged from 9.61–15.21, and 9.08–15.88 %, respectively (Table 1). With increase in extraction time from 40 to 90 min, oil yield extracted using hexane increase significantly ($p < 0.05$) for 100 °C extraction temperature. For ethyl acetate, extraction time did not show any significant ($p > 0.05$) increase on oil yield for the entire experimental extraction temperature range. For camelina seed, at the shortest extraction time (40 min), raising the temperature from 80 to 100 °C resulted in significant ($p < 0.05$) increase in ethyl acetate extracted oil yield. At higher extraction time, there was no significant ($p > 0.05$) increase in oil yield with increasing the temperature. Hexane extracted oil yield did not show any significant ($p > 0.05$) change with temperature for the experimental extraction time range. This camelina seed contained a lower amount of oil and due to almost complete extraction of oil at low extraction time and temperature, there was no significant change in oil data at lower extraction time and higher temperature. However, the maximum oil yield was found as 15.21 % for hexane at 120 °C- 90 min, and 15.88 % for ethyl acetate at 100 °C-90 min of extraction temperature—time (Table 1).

Flax seed contained the oil ranged from 11.10–28.38, and 19.19–33.33 % using hexane and ethyl acetate solvents, respectively (Table 1). For hexane extracted oil, with an increase in extraction time from 40 to 90 min, oil yield did not increase significantly ($p > 0.05$) for all experimental extraction temperature. For ethyl acetate extraction, the oil yield increased significantly ($p < 0.05$) as extraction time increased from 65 to 90 min at 100 and 120 °C extraction temperatures, though at lower temperature (80 °C), there was a significant ($p < 0.05$) difference in oil yield values between 40 and 90 min extraction time. Oil extracted from both the solvents increased significantly ($p < 0.05$) with increase in temperature from 80 to 100 °C at 90 min extraction time. At lower range of extraction time, there was no significant ($p > 0.05$) change in hexane extracted oil yield with increase in temperature. However, for ethyl acetate extracted oil, the percentage yield increased significantly ($p < 0.05$) as temperature increased from 80 to 100 °C at 65 min extraction time and from 100 to 120 °C at 40 min of extraction time. The maximum oil yield from flax seed was obtained as 28.38 and 33.33 % at 120 °C for 90 min of extraction time using hexane and ethyl acetate, respectively (Table 1).

Mustard seed had an oil content varying from 19.50 to 27.68 % and 14.39 to 27.36 % for hexane and ethyl acetate extraction, respectively (Table 1). Hexane extracted oil yield did not show any significant ($p > 0.05$) increase with an increase in extraction time from 40 to 90 min for the experimental extraction temperature range. Except at 80 °C, there was a significant ($p < 0.05$) difference in ethyl acetate extracted oil yield for 40 and 90 min of extraction time. For the experimental extraction time range, hexane extracted oil yield was significantly ($p < 0.05$) different for 80 and 120 °C extraction temperatures, whereas oil yield extracted using ethyl acetate showed the same trend for 65

Table 1 Treatment combination effect of independent variables (i.e. temperature and time) on extracted oil yield (%) for the selected seeds (The ASE operating pressure was controlled at 1500 psi throughout the experiments)

Trt. Cond.	Time (min)	Temp (°C)	Canola		Camelina		Flax		Mustard	
			Hexane	Ethyl acetate	Hexane	Ethyl acetate	Hexane	Ethyl acetate	Hexane	Ethyl acetate
1	40	80	21.08 ± 1.22^{a}	25.12 ± 1.71^{a}	9.61 ± 0.59^{a}	9.08 ± 0.72^{a}	11.10 ± 1.55^{a}	19.19 ± 1.29^{a}	19.73 ± 0.57^{ab}	20.54 ± 0.54^{bc}
2		100	29.67 ± 1.59^{bc}	28.39 ± 0.65^{abc}	9.91 ± 0.89^{ab}	14.33 ± 1.24^{b}	22.83 ± 0.83^{cd}	22.59 ± 1.71^{ab}	20.96 ± 0.35^{abc}	18.30 ± 1.16^{ab}
3		120	33.61 ± 0.77^{cd}	27.70 ± 1.42^{ab}	13.21 ± 0.95^{abcd}	15.18 ± 1.44^{b}	24.55 ± 1.77^{cd}	28.33 ± 0.49^{c}	25.29 ± 1.48^{cd}	21.52 ± 1.30^{bc}
4	65	80	27.74 ± 0.79^{b}	26.49 ± 1.74^{a}	10.48 ± 1.23^{abc}	13.02 ± 1.09^{ab}	21.22 ± 1.49^{bc}	22.43 ± 1.22^{ab}	19.50 ± 1.49^{a}	14.39 ± 1.17^{a}
5		100	34.59 ± 1.01^{d}	26.48 ± 1.33^{a}	14.01 ± 1.25^{bcd}	14.09 ± 1.38^{b}	23.66 ± 1.59^{cd}	28.43 ± 0.35^{c}	23.09 ± 0.53^{abcd}	24.85 ± 1.07^{cd}
6		120	35.93 ± 1.12^{d}	32.20 ± 1.73^{bcd}	13.63 ± 1.68^{abcd}	13.96 ± 0.92^{b}	24.03 ± 1.83^{cd}	28.26 ± 1.25^{c}	24.23 ± 1.29^{bcd}	24.39 ± 0.46^{cd}
7	90	80	28.68 ± 1.87^{b}	33.13 ± 0.72^{cd}	11.38 ± 0.58^{abcd}	11.97 ± 1.52^{ab}	16.57 ± 1.28^{ab}	24.75 ± 0.66^{bc}	21.78 ± 1.64^{abc}	21.66 ± 1.21^{bc}
8		100	35.41 ± 1.59^{d}	36.63 ± 1.02^{de}	14.64 ± 0.83^{cd}	15.88 ± 0.87^{b}	24.22 ± 1.63^{cd}	32.69 ± 0.51^{d}	24.29 ± 1.72^{bcd}	24.61 ± 0.56^{cd}
9		120	36.44 ± 0.41^{d}	40.38 ± 1.39^{e}	15.21 ± 1.50^{d}	14.82 ± 0.48^{b}	28.38 ± 0.48^{d}	33.33 ± 1.09^{d}	27.68 ± 0.45^{d}	27.36 ± 1.72^{d}
Soxhlet extraction			42.96 ± 0.71		16.24 ± 0.40		35.62 ± 1.04		28.80 ± 1.32	

Means within a column with different superscripts are significantly different ($p < 0.05$)

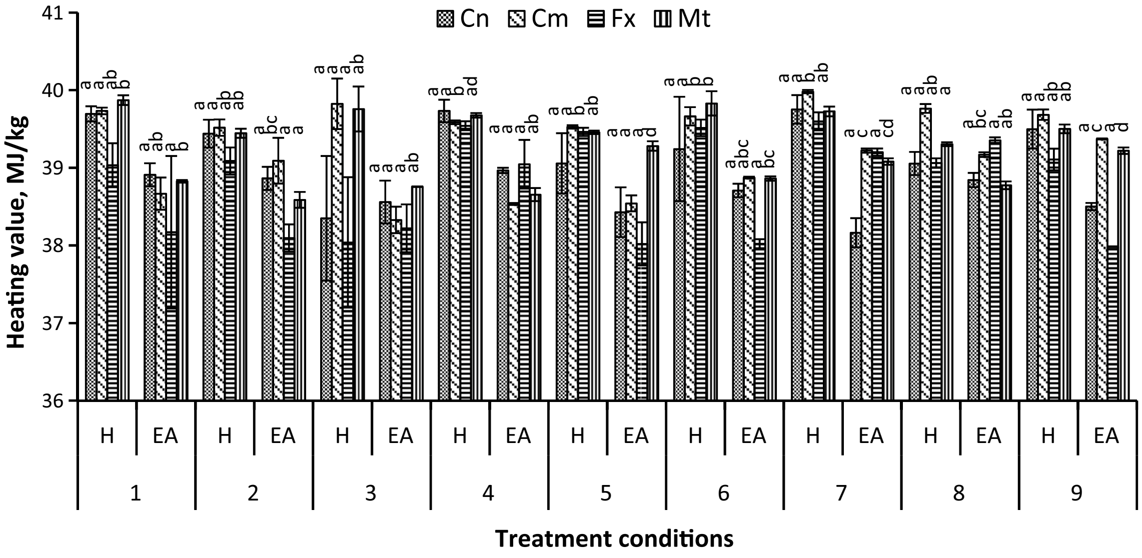

Fig. 1 Treatment effect of independent variables on heating value for all oilseeds and solvents (*H* hexane, *EA* ethyl acetate). Column means within same solvent and oilseeds with different superscripts are significantly different ($p < 0.05$)

and 90 min of extraction time. Mustard seed had maximum oil yields of 27.68 and 27.36 % at 120 °C for 90 min of extraction time using hexane and ethyl acetate, respectively (Table 1).

Among all the above oilseeds, canola had the maximum oil content followed by flax, mustard and camelina. Maximum oil yield was found from all oilseeds at 120 °C extraction temperature and 90 min extraction time. Overall, temperature had a more significant effect on oil yield that extraction time. Ethyl acetate extraction exhibited the oil yield data equivalent to that of hexane for all experimental oilseeds.

Oil yields of all oilseeds extracted with ethyl acetate using ASE were also comparable with those using Soxhlet extraction. Maximum ASE oil yield of canola, camelina, flax and mustard were 6.0, 2.2, 6.4, and 5.0 % less than Soxhlet extracted oil yield, respectively (Table 1).

Effect of Extraction Time and Temperature on Oil Qualities

Heating Value

Heating value for canola seed ranged from 38.35–39.78, and 38.16–38.91 MJ/kg for hexane and ethyl acetate extraction, respectively. Similar data were found by Thompson and He [18]; and Giakoumis [19]. Hexane and ethyl acetate extracted oil did not show any significant ($p > 0.05$) change in heating values with increases in extraction time and temperature (Fig. 1).

For camelina seed, heating values varied from 39.52–39.98, and 38.33–39.37 MJ/kg for hexane and ethyl acetate extracted oils, respectively. Dobre and Jucoane [20] reported a similar value, however, Bernardo *et al.* [21]; Patil *et al.* [22]; and Leung *et al.* [23] found higher heating values of camelina oil. The heating values of oil extracted using hexane did not significantly ($p > 0.05$) change for experimental extraction times and temperatures. Ethyl acetate extracted oil exhibited the significant ($p < 0.05$) increase in heating values with increase in extraction time from 40 min to 90 min at 80 and 120 °C extraction temperature. There was no significant ($p > 0.05$) change in heating values of ethyl acetate extracted oil with increase in extraction temperature from 80 to 120 °C for 65 and 90 min of extraction time (Fig. 1).

Flax oil exhibited heating values ranging from 38.04–39.60, and 37.98–39.35 MJ/kg extracted using hexane and ethyl acetate solvents, respectively. These values are in agreement with the works of Barnwal and Sharma [24]; Demirbas [25, 26]. The heating value of hexane extracted oil only showed the significance ($p < 0.05$) increase at 120 °C extraction temperature when extraction time increased from 40 to 65 min. Increases in extraction temperature did not significantly ($p > 0.05$) change the heating value for all experimental extraction times. Extraction time and temperature had no significant ($p > 0.05$) effect on heating values of flax oil extracted using ethyl acetate (Fig. 1).

The heating values of mustard oil extracted using hexane and ethyl acetate were found to be in the range of 39.30–39.87, and 38.59–39.28 MJ/kg, respectively which are in accordance with values reported by Demirbas [25]; Azad *et al.* [27]; and Sanjid *et al.* [28]. The effects of extraction time and temperature on heating values of hexane

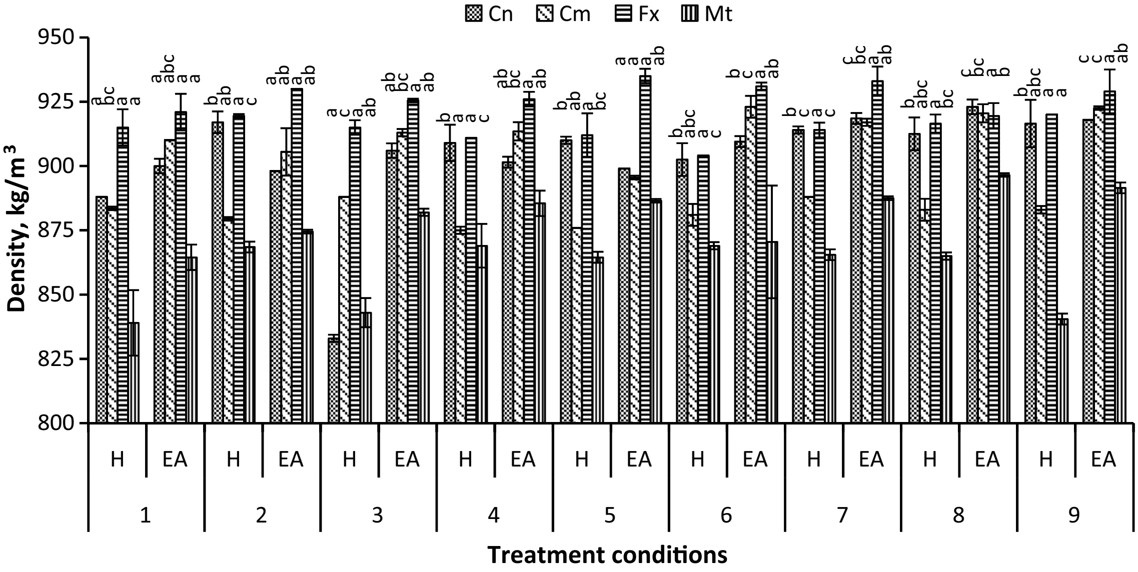

Fig. 2 Treatment effect of independent variables on density for all oilseeds and solvents (*H* hexane, *EA* ethyl acetate). Column means within same solvent and oilseeds with different superscripts are significantly different ($p < 0.05$)

extracted oil were not found to be significant ($p > 0.05$). For ethyl acetate, there was a significant ($p < 0.05$) increase in heating values when extraction time increased from 65 to 90 min at 80 and 120 °C extraction temperatures. An increase in temperature form 100 to 120 °C showed a significant ($p < 0.05$) increase in heating value of ethyl acetate extracted mustard oil at 90 min of extraction time (Fig. 1).

The average heating values of all the above oilseeds were almost the same. The average heating values for hexane extracted oils for all oilseeds were almost 2 % higher than that of ethyl acetate. Higher heating values of all oilseeds were obtained at 90 min extraction time and in the range of extraction temperature from 80 to 120 °C.

Density

Densities of hexane and ethyl acetate extracted canola oil ranged from 833–917, and 898–923 kg/m^3, respectively. Lang *et al.* [29]; Latif *et al.* [17]; and Giakoumis [19] reported similar results. At 120 °C extraction temperature, the density of hexane extracted oil increased significantly ($p < 0.05$) with increase in extraction time from 40 to 65 min, whereas density was decreased significantly ($p < 0.05$) with an increase in temperature form 100 to 120 °C at 40 min of extraction time. For ethyl acetate extracted oil, density increased significantly ($p < 0.05$) with increase in extraction time from 65 to 90 min for experimental extraction temperature range. As temperature increased from 100 to 120 °C at 65 min time of extraction, density of oil increased significantly ($p < 0.05$) for ethyl acetate extraction (Fig. 2).

Camelina oil had densities varying from 875–888, and 895.5–923 kg/m^3 for hexane and ethyl acetate extraction, respectively which are in accordance with the values reported by Bernardo *et al.* [21]; Patil *et al.* [22]; Leung *et al.* [23]; and Dobre and Jucoane [20]. There was no significant ($p > 0.05$) change in density of hexane extracted oil when the extraction time increased from 40 to 90 min in the experimental extraction temperature range. There was only a significant ($p < 0.05$) increase found in density with an increase in extraction temperature from 100 to 120 °C at 40 min of extraction time. For ethyl acetate extraction, no significant ($p > 0.05$) change was observed in oil density with increased extraction time, and temperature from 40 to 90 min, and from 80 to 120 °C, respectively (Fig. 2).

Hexane and ethyl acetate extracted flax oil showed density values ranging from 904–920, and 919.5–935 kg/m^3, respectively which are in agreement with the values reported by Srivastava and Prasad [30]; Lang *et al.* [29]; Barnwal and Sharma [24]; and Demirbas [25, 31]. For both the solvents extracted oils, there was no significant ($p > 0.05$) change in density observed with increases in extraction time and temperature (Fig. 2).

For mustard, hexane and ethyl acetate extracted oils had densities varying from 839–869, and 864.5–896.5 kg/m^3, respectively. However, Demirbas [25]; Sivaramakrishnan and Ravikumar [32]; Azad *et al.* [27]; Tulip and Radha [33]; Sanjid *et al.* [28]; and Zahir *et al.* [34] reported the density of flax oil to be more than 925 kg/m^3. Hexane extracted oil density significantly ($p < 0.05$) increased with an increase in extraction time 40 to 65 min at 80 and 120 °C extraction temperatures. There was a significant

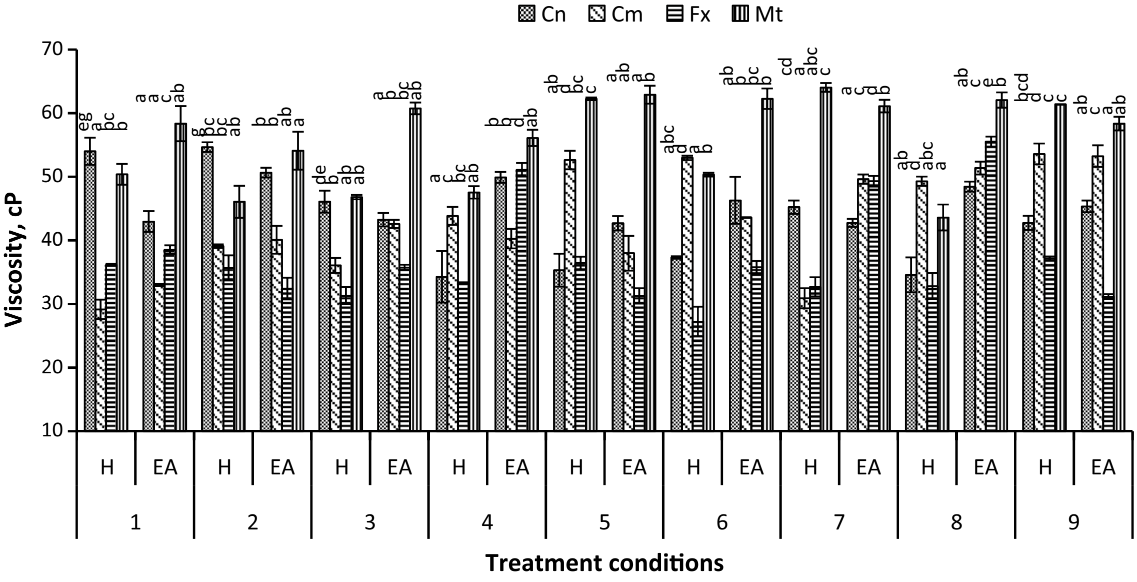

Fig. 3 Treatment effect of independent variables on viscosity for all oilseeds and solvents (*H* hexane, *EA* ethyl acetate). Column means within same solvent and oilseeds with different superscripts are significantly different ($p < 0.05$)

($p < 0.05$) drop in density values when extraction temperature increased from 100 to 120 °C at 40 and 90 min of extraction time. There was no significant ($p > 0.05$) effect of changing the temperature from 80 to 120 °C on oil density at experimental extraction time range. For ethyl acetate extracted oil, the density did not change significantly ($p > 0.05$) with increase in extraction time and temperature. However, the minimum value at 40 min and 80 °C was significantly ($p < 0.05$) differ than maximum value at 90 min and 100 °C (Fig. 2).

Flax seed had the maximum density followed by canola, camelina and mustard. Oil extracted using ethyl acetate had slightly higher density values than those of hexane extracted oils for all oilseeds. Maximum densities of all oilseeds were observed at an extraction temperature ranging from 100–120 °C for a 90-min extraction time.

Viscosity

Hexane and ethyl acetate extracted oils had viscosities ranging from 34.27–54.66, and 42.70–50.65 cP, respectively. The value of hexane extracted oil was found to be in accordance with Lang *et al.* [29]; and Fasina and Colley [35]. With an increase in extraction time from 40 to 65 min, the viscosity of hexane extracted oil decreased significantly ($p < 0.05$) at all experimental extraction temperatures. At 40 min of extraction time, the viscosity decreased significantly ($p < 0.05$) with an increase in extraction temperature from 100 to 120 °C, whereas the same result was observed at 90 min of extraction time when the temperature was increased from 80 to 100 °C. Viscosity of ethyl acetate extracted oil significantly ($p < 0.05$) decreased when the extraction time was increased from 40 to 65 min at a 100 °C extraction temperature. There was no significant ($p > 0.05$) change in viscosity with increasing the temperature from 80 to 120 °C for the experimental extraction time range (Fig. 3).

Camelina oil exhibited viscosities varying from 29.16–53.58, and 32.99–53.24 cp for hexane and ethyl acetate extraction, respectively. Bernardo *et al.* [21] reported similar results. Hexane extracted oil viscosity increased significantly ($p < 0.05$) with an increase in extraction time from 40 to 65 min in the experimental extraction temperature range. However, the value decreased significantly ($p < 0.05$) at 80 °C extraction temperature when the time was increased from 65 to 90 min, while for other two extraction temperatures, there was no significant ($p > 0.05$) change observed. With an increase in extraction temperature from 80 to 100 °C, the viscosity increased significantly ($p < 0.05$) within the experimental extraction time range. For ethyl acetate extracted oil, viscosity increased significantly ($p < 0.05$) with increase in extraction time from 65 to 90 min at all experimental temperatures. At 40 min extraction time, viscosity significantly ($p < 0.05$) increased when extraction temperature increased from 80 to 100 °C (Fig. 3). The increase in viscosity was due to extraction of some other components (cloudy) with oil from oilseed.

The viscosities of flax oil ranged from 27.23–37.19, and 31.16–55.52 cP for hexane and ethyl acetate solvent extraction, respectively. Lang *et al.* [29] reported the viscosity of flax oil as 22.40 cP. At 120 °C extraction temperature, the viscosity of hexane extracted oil increased significantly

($p < 0.05$) with an increase in extraction time from 65 to 90 min. However, viscosity decreased significantly ($p < 0.05$) when extraction temperature increased from 100 to 120 °C for 65 min of extraction time. For ethyl acetate extracted oil, viscosity significantly ($p < 0.05$) decreased when the extraction time increased from 65 to 90 min at 120 °C extraction temperature. With increase in extraction temperature from 80 to 120 °C, the viscosity decreased significantly ($p < 0.05$) for all experimental extraction times (Fig. 3).

Mustard oil extracted using hexane and ethyl acetate had viscosities varied from 43.61–64.03, and 54.10–62.90 cP, respectively. These values are in agreement with those of Azad *et al.* [27]. Increasing extraction time from 40 to 90 min, the viscosity of hexane extracted oil significantly ($p < 0.05$) increased at 80 and 120 °C extraction temperatures. There was no significant ($p > 0.05$) change in viscosity at 100 °C with increases in time. With an increase in temperature from 80 to 120 °C, there was no significant

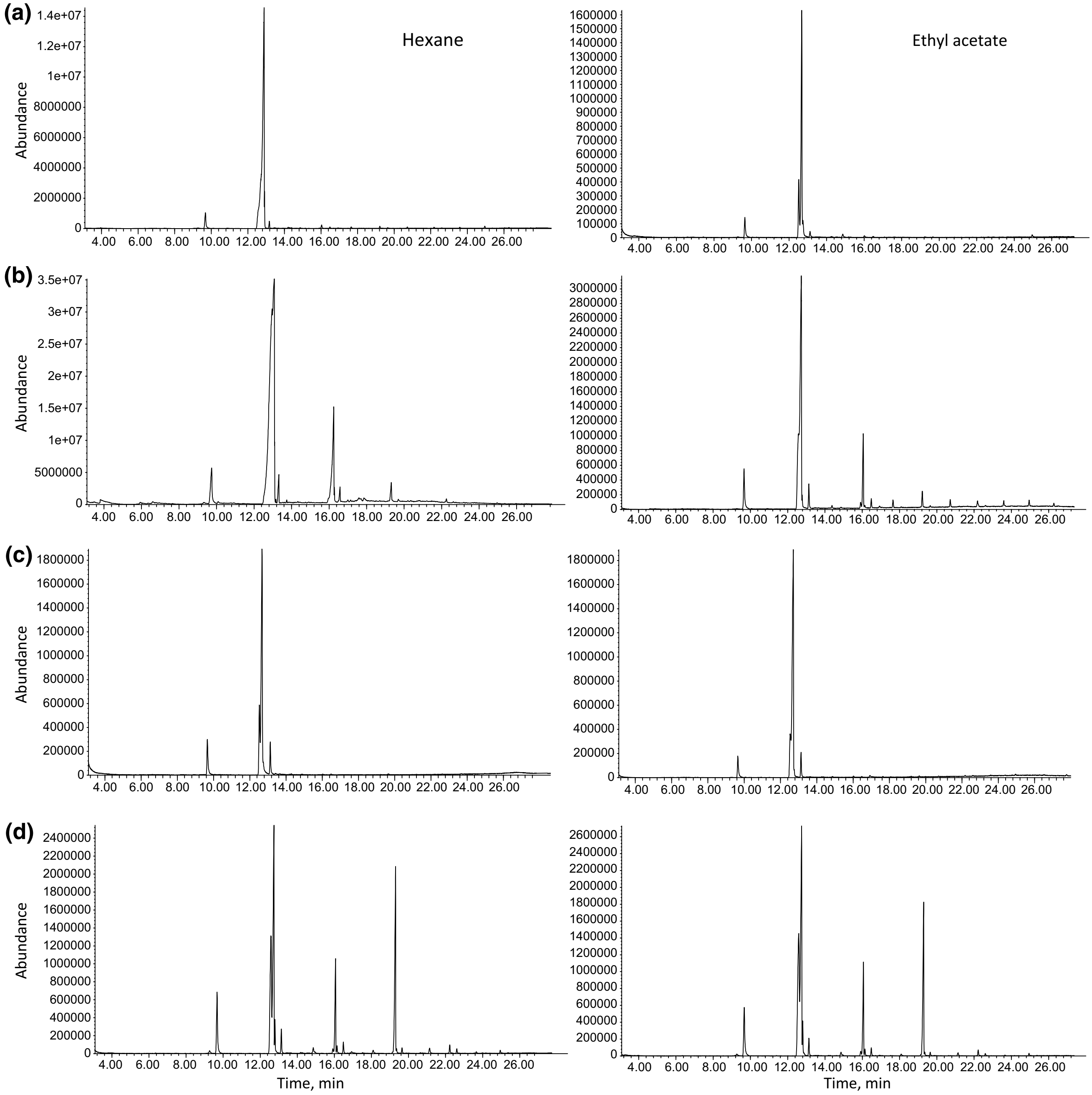

Fig. 4 GC-MS chromatogram of **a** canola, **b** camelina, **c** flax, and **d** mustard for both the solvents at 100 °C and 90 min extraction condition

Table 2 Centesimal composition of fatty acids of all oilseeds for both the solvents

Fatty acids	Carbon number	Canola		Camelina		Flax		Mustard	
		Hexane	EA	Hexane	EA	Hexane	EA	Hexane	EA
Palmitic	16:0	4.18	4.37	6.91	7.26	4.43	4.16	3.91	3.92
Linoleic	18:2	21.22	21.15	23.42	22.79	15.99	15.57	23.09	24.66
Linolenic	18:3	8.97	9.33	50.11	49.21	54.93	54.19	29.96	26.37
Oleic	18:1	64.04	63.67	17.28	18.58	20.83	22.69	40.80	42.92
Stearic	18:0	1.59	1.48	2.28	2.16	3.82	3.39	2.24	2.13

EA ethyl acetate

($p > 0.05$) change observed in viscosity within the experimental extraction time range. However, the minimum viscosity was found at 100 °C and 90 min extraction time. Mustard oil extracted with ethyl acetate only exhibited a significant ($p < 0.05$) increase in viscosity when the extraction time was increased from 40 to 65 min at 100 °C temperature. No significant ($p > 0.05$) change was observed in viscosity with increases in temperature for all experimental extraction times (Fig. 3).

The lowest viscosity was found for flax seed followed by camelina, canola and mustard. Only for flax oilseeds, oil extracted using ethyl acetate showed higher viscosity as compare to that of hexane extracted oil. Minimum oil viscosities for all oilseeds for both the solvents were observed at a range of extraction temperatures from 80 to 100 °C for 90 min of extraction time.

FFA Profile

Fee fatty acid profiles for all oilseeds at 100 °C and 90 min are given in Fig. 4. Five free fatty acids were identified in all oilseeds. From the observation of peak heights and areas of the chromatogram, it was found that extracted oils from canola and mustard oilseed contained maximum quantity of oleic acid (monounsaturated fatty acid), whereas, camelina and flaxseeds oils were rich in linolenic acid as a polyunsaturated fatty acid regardless the extraction solvents (Table 2). Similar reports were found by Giakoumis [19]; Hong *et al.* [36] for canola, Dobre and Jucoane [20]; Rosie *et al.* [37]; Waraich *et al.* [38] for camelina, Sanford *et al.* [39]; Singh and Singh [40] for flax seed, and Bannikov [41]; Tulip and Radha [33] for mustard.

Unsaponifiable Matter

The effect of extraction temperature and time on unsaponifiable matter of canola, camelina, flax and mustard oils is shown in Table 3. For hexane extracted canola and camelina oil, for 40 min of extraction time, unsaponifiable matter was significantly ($p < 0.05$) reduced with increase in temperature from 80 to 120 °C, while for longer extraction time, extraction temperature did not have any significant ($p > 0.05$) effect on unsaponifiable matter. Similar results were found for ethyl acetate extracted canola and camelina oil. The lowest unsaponifiable matter was found at 120 °C-90 min condition for both the solvent extracted canola and camelina oils. At lower extraction temperature, with increase in extraction time, unsaponifiable matter in hexane and ethyl acetate extracted flax oil significantly ($p < 0.05$) decreased, whereas, at higher extraction temperature, there was no significant ($p > 0.05$) effect of extraction time on unsaponifiable matter of flax oil extracted using hexane and ethyl acetate. Unsaponifiable matter of hexane extracted mustard oil was not significantly ($p > 0.05$) effected by extraction time and temperature, while, for higher extraction time, unsaponifiable matter of ethyl acetate extracted mustard oil was significantly ($p < 0.05$) reduced with increase in extraction temperature. The lowest unsaponifiable matter was found at a higher extraction time and temperature for all the oils extracted using both solvents. The values of unsaponifiable matter of all oils were found in range reported by Choo *et al.* [42]; Latif *et al.* [17]; Czaplicki *et al.* [43]; El-Beltagi *et al.* [44]; and The *et al.* [45].

Comparison of Solvents

The relevant properties of ethyl acetate as compared to *n*-hexane as solvent are listed in Table 4. Ethyl acetate has almost similar molecular weights, boiling points, enthalpy of vaporization, and surface tension to *n*-hexane to substitute it. From the safety point of view, ethyl acetate has higher flash points than *n*-hexane, so it is less flammable and hazardous. As far as cost is concerned, ethyl acetate is 33 % cheaper per unit as compare to *n*-hexane. The major drawback of using ethyl acetate is its higher viscosity, density and dielectric point. But in ASE, solvents were used at higher temperatures than their boiling points. Under this condition, the ethyl acetate would be in a subcritical state since the working pressure (1500 psi) was above

Table 3 Treatment combination effect of independent variables (i.e. temperature and time) on unsaponifiable matter (%) for the selected seeds

Trt. Cond.	Time (min)	Temp (°C)	Canola		Camelina		Flax		Mustard	
			Hexane	Ethyl acetate	Hexane	Ethyl acetate	Hexane	Ethyl acetate	Hexane	Ethyl acetate
1	40	80	0.69 ± 0.05^{a}	0.57 ± 0.03^{ab}	0.70 ± 0.08^{ab}	0.56 ± 0.02^{ab}	0.79 ± 0.14^{a}	0.59 ± 0.06^{a}	0.79 ± 0.02^{a}	0.58 ± 0.01^{ab}
2		100	0.58 ± 0.03^{b}	0.54 ± 0.01^{bc}	0.59 ± 0.03^{c}	0.59 ± 0.03^{a}	0.47 ± 0.03^{bc}	0.47 ± 0.07^{abc}	0.66 ± 0.09^{ab}	0.59 ± 0.05^{ab}
3		120	0.49 ± 0.01^{cd}	0.46 ± 0.02^{de}	0.65 ± 0.08^{ab}	0.42 ± 0.03^{bc}	0.42 ± 0.03^{bc}	0.36 ± 0.00^{cd}	0.60 ± 0.02^{b}	0.59 ± 0.02^{ab}
4	65	80	0.55 ± 0.01^{bc}	0.56 ± 0.03^{abc}	0.78 ± 0.08^{a}	0.63 ± 0.07^{a}	0.53 ± 0.03^{b}	0.51 ± 0.04^{ab}	0.62 ± 0.08^{ab}	0.67 ± 0.05^{a}
5		100	0.50 ± 0.01^{bcd}	0.64 ± 0.03^{a}	0.63 ± 0.05^{ab}	0.43 ± 0.05^{bc}	0.44 ± 0.04^{bc}	0.35 ± 0.00^{cd}	0.67 ± 0.00^{ab}	0.45 ± 0.04^{c}
6		120	0.54 ± 0.01^{bcd}	0.57 ± 0.02^{abc}	0.62 ± 0.04^{abc}	0.38 ± 0.03^{c}	0.39 ± 0.03^{c}	0.36 ± 0.02^{cd}	0.64 ± 0.02^{ab}	0.49 ± 0.01^{bc}
7	90	80	0.52 ± 0.02^{bcd}	0.50 ± 0.01^{bcd}	0.66 ± 0.08^{ab}	0.42 ± 0.02^{bc}	0.67 ± 0.05^{a}	0.45 ± 0.01^{bc}	0.73 ± 0.05^{ab}	0.63 ± 0.03^{a}
8		100	0.54 ± 0.02^{bcd}	0.49 ± 0.01^{cd}	0.60 ± 0.04^{abc}	0.36 ± 0.02^{c}	0.45 ± 0.03^{bc}	0.28 ± 0.01^{d}	0.58 ± 0.02^{b}	0.64 ± 0.04^{a}
9		120	0.46 ± 0.01^{d}	0.39 ± 0.01^{e}	0.53 ± 0.01^{bc}	0.35 ± 0.04^{c}	0.36 ± 0.00^{c}	0.31 ± 0.01^{d}	0.56 ± 0.01^{b}	0.42 ± 0.02^{c}

Means within a column with different superscripts are significantly different ($p < 0.05$)

Table 4 Relevant properties of *n*-hexane and ethyl acetate

Properties	*n*-hexane	Ethyl acetate
Molecular weight (g/mol)	86.18	88.11
Density 25 °C (g/ml)	0.655	0.897
Boiling point (°C)	68.5	77.1
Flash point (°C)	−26.0	−4.0
Viscosity 25 °C (cP)	0.29	0.43
Enthalpy of vaporization (kJ/mol)	28.85	31.94
Surface tension 25 °C (dyne/cm)	17.91	23.75
Dielectric constant 25 °C	1.88	6.02
Cost ($/L)	95.97	64.29

Source Sigma-Aldrich

the critical pressure of that solvent (557.0 psi). Therefore, ethyl acetate would have a smaller dielectric constant and, hence, its polarity would also be decreased [46, 47]. It may be possible to determine the dielectric constant of ethyl acetate that decreases considerably, from 6.02 under normal conditions to 3.19 under these extraction conditions [48, 49]. Wissam *et al.* [50] reported that the ethyl acetate at 90 °C could not extract the polar compounds from pomegranate peel at 90 °C due to its lower dielectric constant at that temperature. Therefore, ethyl acetate can be used as a solvent to extract the oil from oilseeds without extracting polar compounds under given extraction conditions.

Conclusions

From the results from this study it can be concluded that the yield and quality (heating value, density, viscosity, free fatty acid profile, and unsaponifiable matter) of oil removed from different oilseeds (canola, camelina, flax, mustard) using ethyl acetate are almost equivalent to those of the oils removed using hexane. Extraction time and temperature of 90 min, and 100 °C, respectively can be recommended for oil extraction from selected oilseeds using ethyl acetate. Though the densities and viscosities of ethyl acetate extracted oils were slightly higher than those of the hexane extracted oils, ethyl acetate can be substituted for hexane in oilseed processing plants with limited retrofitting of the equipment. Ethyl acetate also offers a potential and cheaper alternative to hexane for oilseed processing in terms of human and environmental safety.

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