

Comparative analysis of physico-chemical properties and fatty acid composition of linseed (*Linum usitatissimum* L.) oils of Indian accessions

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ABSTRACT

The aim of the current study was to investigate the variation in oil content, fatty acid composition, and physico-chemical characteristics of different accessions of linseed oil (*Linum usitatissimum* L.). Significant variations in fatty acid composition, oil content and physico-chemical properties were observed in linseed accessions, among them, unsaturated and saturated fatty acid compositions were ranged from 79.23–86.66% to 12.61–19.99%, respectively, in total fatty acid compositions. The highest content of alpha-linolenic acid (ALA) was found to be major in BHU-A (53.39%) accession followed by BHU-B (53.14%) accessions, whereas the lowest one was found in the accession IC 564631 (38.07%). The current investigation found varying results for the physico-chemical characteristics of linseed oil, such as Iodine value (173.39–178.83 g Iodine/100 g), acid value (2.10–3.04 mg KOH/g), saponification value (192.16–193.49 mg KOH/g), peroxide value (1.52–2.12 meq/Kg), refractive index (1.471–1.472 RI at 40°C), and viscosity (23.37–27.95 at 40°C (cSt)). The findings of our current investigation revealed that linseed oil is a valuable source of ALA which could be potential applications in the food and pharmaceutical industries. Therefore, comparative and characterization analysis of different linseed accessions aids in selecting a good quality linseed oil and provides new breeding resources for linseed accessions.

1. INTRODUCTION

Linseed (*Linum usitatissimum* L.), generally called flaxseed, is a self-pollinating, diploid, herbaceous plant species belonging to the family of Linaceae [1,2]. Flaxseed/linseed is cultivated for two key reasons: Fiber and seed oil. The fiber acquired from the stems is weaved into linen textiles [3]. Linseed crop is cultivated in Kazakhstan, Canada, Russian Federation, China, India, USA, and Ethiopia. Linseed is the most significant oilseed crop in India, but it is mostly a rainfed crop cultivated by farmers with minimal resources. India ranks sixth place globally from the perspective of linseed production, yielding 174,000 tonnes from 320,000 hectares of agricultural land. However, India's average productivity (534 kg/ha) is significantly lower than the global average (1053 kg/ha) [4]. Linseeds provide specific nutritional advantages such as Omega-3 fatty acids, significant content of lignan,

and mucilage gums, making them a desirable commodity in the food industry [5,6].

Linseed is an important profitability oilseed crop [7,8] which is regaining prominence from its conventional utilization as a primary component in the manufacturing of oil due to the stated nutritional advantages of omega-3 fatty acids as well as its extraordinarily greater level of the alpha linolenic acid (ALA). Intake of linseeds is very useful for human and animal health, additionally, they comprise a number of bioactive phenolic compounds which show biological activity inclusive of antiradical, antioxidant, antimicrobial, and anticancer consequences [9,10]. However, flaxseed oil's high ALA concentration makes it particularly vulnerable to oxidation, resulting in rapid quality degradation. Lukaszewicz *et al.* [11] stated that linseed oil is frequently added with antioxidants and preserved in dark containers following cold wrenching out to minimize the quick formation of peroxidation.

2. MATERIALS AND METHODS

Two linseed accessions were acquired from Banaras Hindu University, Varanasi, Uttar Pradesh and ten linseed accessions from NBPGR, New Delhi, India [Table 1]. HiMedia Laboratories Pvt. Ltd., provided

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Table 1: Details of the linseed samples analyzed in the present study.

Accessions	Source of collection of linseed accessions
BHU-A	Banaras Hindu, University, Varanasi, Uttar Pradesh
BHU-B	Banaras Hindu, University, Varanasi, Uttar Pradesh
IC-564616	NBPGR, New Delhi
IC-564622	NBPGR, New Delhi
IC-564631	NBPGR, New Delhi
IC-564592	NBPGR, New Delhi
IC-564630	NBPGR, New Delhi
IC-564660	NBPGR, New Delhi
IC-564585	NBPGR, New Delhi
IC-564676	NBPGR, New Delhi
IC-564624	NBPGR, New Delhi
IC-564605	NBPGR, New Delhi

all of the chemicals and solvents utilized in the experiment, which were of analytical quality.

2.1. Extraction of Seed Oils

50 g of linseeds were finely pulverized in a grinder and transferred to the thimble of the Soxhlet extraction unit [12]. A sufficient amount of petroleum ether was used as a solvent to completely wet the powder and the mixture was then heated to (40–60°C) temperature for around 10 h. The oil and solvent mixture were removed after 10 h, and the solvent was evaporated in a vacuum dryer at 40°C using a rotary evaporator. The extracted oil was then stored at 4°C in glass bottles beneath nitrogen blanket for further physico-chemical assessment. The following equation was used to determine the oil percentage.

$$\text{Percentage of oil} = ([W_b - W_a] \times 100) / \text{Weight of the sample taken}$$

Where, W_a denotes the Wt of the blank flask and W_b = The Wt of the flask with the extracted oil.

2.2. Determination of the Fatty Acid Composition

For the conversion of linseed oil to fatty acid methyl esters (FAME) 0.1 g (100 mg) of linseed oil, samples were tested over 2 h at 55°C using 2% sulfuric acid (H_2SO_4) in 20 ml of methanol [13]. TLC is used to monitor the development of methanolysis using a solvent mixture comprising hexane: ethyl acetate (90:10, v/v). The esters have been converted into ethyl acetate, neutralized using water then dried on anhydrous sodium sulfate (Na_2SO_4). To get fatty acid methyl esters, the ethyl acetate samples were condensed subsequently by utilizing a rotary evaporator. The fatty acid content of the transformed fatty acid methyl esters was determined using a gas chromatography-flame ionization detector (GC-FID). The amount and spotting of fatty acids in linseed oil were evaluated using a conventional supelco 37 FAME component. GC using an FID investigation was carried out utilizing an Agilent Technologies 6890N Network GC instrument furnished by a DB-225 (Part No.122-2232), capillary column (25m × 0.25 mm × i.d., film compactness, 0.25 m), and a Flame Ionization Detector. Nitrogen gas was employed as mobile phase (1 ml/min flow rate). The input temperature was controlled at 250°C. The temperature of the detector was fixed at 270°C. The column remained kept at a temperature of 160°C for 2 min before being configured to the terminal temperature of 230°C at a rate of 5°C/min for 14 min. The holding duration of fatty acids was compared to an authorized reference to identify them. The composition of fatty acids methyl esters (FAMES) was quantified by incorporating the FID peak

region with a correction factor (internal normalization approach). Single component correction factors were estimated employing a specified composition of methyl esters (Supelco 37 comp. FAME mix). All gas chromatogram analyses were performed three times, as well as the mean results were presented in the current study.

2.3. Saponification Value (SV)

The saponification number indicates the quantity of alkali required to saponify a certain amount of the test material. This is usually explained as the number of milligrams of potassium hydroxide (KOH) needed to saponify 1 g of the test material. Official method of AOCS cd 3b-76 [14] was used to determine the SV of linseed oils. 5 g of test samples was saponified in a water bath for about 1 h using alcoholic potassium hydroxide. This solution was titrated to 0.5N HCL utilizing 1% phenolphthalein as a reagent. The SV was calculated using the following formula

$$\text{Saponification value (SV)} = \frac{(B - S) \times N \times 56.1}{W}$$

Where, N = Normality of HCL; W = Wt of test samples; B = Volume of HCL in ml titrated against blank; S = Volume of HCL in ml titrated against test sample.

2.4. Iodine Value (IV)

Iodine level is an evaluation of unsaturated fatty acids and is measured in centigrams of iodine consumed each 1 gram of material. The AOCS Cd1-25 [15], the approved procedure is used to calculate the iodine value. About 0.2 g of the test sample was solubilized in 15 ml of carbon tetrachloride and Wijis solution (25 ml) was mixed then stored in darkness for 1 h. 20 ml solution of KI (potassium iodide) along with distilled water (10 ml) was mixed into the mixture. A 0.1N $Na_2S_2O_3$ (sodium thiosulfate) was used to titrate the sample. The iodine value (IV) was determined by the following equation:

$$\text{Iodine Value (IV)} = \frac{(B - S) \times N \times 12.69}{W}$$

Where, N = Normality of $Na_2S_2O_3$ W = Wt of sample in grams; B = Volume of sodium thiosulfate (ml) titrated against blank; S = Volume of sodium thiosulfate (ml) titrated against oil sample.

2.5. Peroxide Value (PV)

The PV was determined using an AOCS-approved technique cd 8-53 [16]. 5 g quantity of test sample was solubilized in an acetic acid and chloroform mixture; then, a saturated potassium iodide (KI) was applied to the solution. The quantity of iodine released by KI from oxidation reaction of peroxides contained within the oil was estimated by titration with 0.1N $Na_2S_2O_3$. Subsequently, Titration was carried out on the blank sample.

$$\text{Peroxide value (IV)} = \frac{(S - B) \times N \times 1000}{W}$$

Where B = Quantity of $Na_2S_2O_3$ utilized for blank, W = Wt of the sample, S = Amount of $Na_2S_2O_3$ observed by the sample and N = Normality of $Na_2S_2O_3$.

2.6. Acid Value (AV)

It is the amount of potassium hydroxide (KI) needed to neutralize the free acids having in 1 gram of sample. As per the standard AOCS

official method Cd 3d-63 [17], acid level was estimated in a volumetric flask, approximately 2 g of sample was taken and 125 ml of the solvent combination was added (1:1 isopropyl alcohol and toluene) and the phenolphthalein indicator was added. This solution was titrated against 0.1N KOH and the amount of acid was determined by utilizing following equation

$$\text{Acid Value (AV)} = \frac{N \times 56.1 \times B.R}{W}$$

Where B.R = Burette reading; N = Normality of standard alkali; W = Wt of the sample.

2.7. Viscosity

The viscosity of a fluid is usually defined as its friction toward movement. Viscosity observations were taken at 40°C and 100°C utilizing standardized Cannon-Fenske viscometer linear units in a Cannon Constant Temperature Viscosity Bath (Cannon Instrument Co., U.S.A). The standard ASTM D445 [18] technique was used to estimate the viscosity.

Kinematic viscosity (cSt) = Viscometer constant (cSt/s) × time (s)

2.8. Refractive Index (RI)

The Refractive Index was determined by AOCS official method Tp 1a-64 [19], using an automatic digital refractometer (Instrument Model No, RFm 870). All of the readings were obtained at 40°C.

2.9. Statistical Analysis

An analysis of variance (ANOVA) was carried out utilizing SPSS software version 28.0. Statistically significant differences ($P \leq 0.05$) in means were determined using Tukey's multiple comparison test. Values were expressed as mean ± SD of three replicates ($n = 3$).

3. RESULTS AND DISCUSSION

3.1. Oil Content and Fatty Acid Profile

The percentage of oil content and fatty acid profile of the studied linseed accessions is tabulated in Tables 2 and 3. Oil content ranged from 30.52% to 46.58%, representing a significant difference among accessions ($P < 0.05$). The BHU-A accession had the highest oil yield (46.58%), whereas the IC-564630 accession had the lowest (30.52%) oil yield. In the present investigation, we reported the highest seed oil content in Indian accessions of linseed in comparison with Shivaraj *et al.* [20] who reported linseed oil percentages varied from 29.4 to 42.6% and Wakjita *et al.* [21] who obtained that the range of seed oil content from Ethiopian genotypes varies from 29.1 to 35.9%. The fatty acid profile of 12 Indian linseed accessions was determined using GC-FID, and the results were presented in a table [Table 2, Figures 1 and 2]. Myristic acid (14:0), palmitic acid (16:0), stearic acid (18:0), archidic acid (20:0), Behenic acid (22:0), and lignoceric acid (24:0) are saturated fatty acids (SFA) which were found to be in the range of 12.61%–19.99 % in all 12 linseed oils. Unsaturated fatty acids predominated (79.23-86.66%) in linseed accessions compared to saturated fatty acids, with higher poly unsaturated fatty acid (PUFA) concentrations [Figure 3]. The PUFA like ALA (18:3), which belongs to the omega (ω^3) fatty acid family and linoleic acid (18:2, ω^6) were found to be in the range of 49.62%–63.78%. The accession BHU-A had the highest PUFA content (63.78%), followed by BHU-B (63.44%), whereas IC-564631 accessions had the lowest (49.62%). The mono

Table 2: Comparison of fatty acid composition and oil content of linseed oils.

Fatty acids	BHU-A	BHU-B	A1	B2	C3	D4	E5	F6	G7	H8	I9	J10
Myristic acid (14:0)	0.05±0.00 ^a	0.00±0.00 ^b	0.09±0.02 ^a	0.05±0.00 ^a	0.00±0.00 ^b	0.00±0.00 ^b	0.00±0.00 ^b	0.08±0.01 ^a	0.00±0.00 ^b	0.00±0.00 ^b	0.05±0.05 ^a	0.08±0.00 ^a
Palmitic acid (16:0)	6.27±0.00 ^g	6.30±0.01 ^{ef}	6.14±0.00 ^{ij}	6.03±0.00 ^k	9.61±0.01 ^a	6.21±0.01 ^{gh}	8.36±0.03 ^b	6.52±0.00 ^d	6.16±0.02 ^{hi}	6.36±0.01 ^e	6.08±0.00 ^h	7.25±0.00 ^e
Hexadecenoic acid (16:1)	0.19±0.00 ^f	0.29±0.04 ^{def}	0.28±0.00 ^{ef}	0.21±0.00 ^{ef}	0.49±0.05 ^{bcd}	0.37±0.00 ^{de}	0.54±0.08 ^{bc}	0.42±0.00 ^{cd}	0.65±0.07 ^{ab}	0.80±0.00 ^a	0.18±0.000 ^f	0.49±0.01 ^{bcd}
Stearic acid (18:0)	5.74±0.00 ^k	5.77±0.00 ^j	6.56±0.00 ^h	6.98±0.00 ^g	9.20±0.00 ^b	7.21±0.00 ^f	9.92±0.01 ^a	7.57±0.00 ^e	7.33±0.00 ^e	7.41±0.00 ^d	6.96±0.00 ^g	6.27±0.00 ^g
Oleic acid (18:1)	22.54±0.01 ^k	22.66±0.01 ^j	24.32±0.00 ^f	26.25±0.01 ^d	28.93±0.01 ^a	25.23±0.04 ^e	28.41±0.01 ^b	26.34±0.02 ^e	26.17±0.01 ^d	24.77±0.02 ^f	23.87±0.00 ^h	23.28±0.00 ⁱ
Linoleic acid (18:2)	10.39±0.00 ^b	10.30±0.01 ^b	11.49±0.00 ^e	11.18±0.00 ^f	11.54±0.00 ^{de}	11.73±0.01 ^{cd}	10.90±0.10 ^g	11.60±0.00 ^{de}	12.10±0.00 ^b	11.26±0.07 ^f	11.85±0.01 ^e	12.70±0.01 ^a
Alpha-Linolenic acid (18:3)	53.39±0.02 ^a	53.14±0.03 ^b	49.51±0.05 ^e	47.77±0.04 ^e	38.07±0.02 ⁱ	47.56±0.02 ^{ef}	39.90±0.01 ^h	45.69±0.02 ^g	45.69±0.00	47.44±0.12 ^f	49.53±0.01 ^e	48.35±0.01 ^d
Arachidic acid (20:0)	0.26±0.00 ^g	0.26±0.00 ^g	0.32±0.00 ^{ef}	0.32±0.00 ^{ef}	0.70±0.00 ^a	0.34±0.01 ^e	0.53±0.00 ^b	0.42±0.00 ^d	0.47±0.01 ^e	0.47±0.01 ^e	0.30±0.01 ^f	0.39±0.001 ^d
Eicosenoic acid (20:1)	0.14±0.02 ^b	0.17±0.02 ^{ab}	0.20±0.00 ^{ab}	0.16±0.02 ^{ab}	0.19±0.00 ^{ab}	0.16±0.00 ^{ab}	0.22±0.10 ^{ab}	0.26±0.04 ^a	0.16±0.02 ^{ab}	0.17±0.00 ^{ab}	0.17±0.01 ^{ab}	0.16±0.01 ^{ab}
Behenic acid (22:0)	0.18±0.00 ^{ef}	0.19±0.00 ^{de}	0.21±0.00 ^{cd}	0.21±0.01 ^{cd}	0.26±0.01 ^a	0.23±0.01 ^b	0.25±0.00 ^a	0.22±0.01 ^{bc}	0.25±0.01 ^a	0.21±0.00 ^{cd}	0.19±0.00 ^{de}	0.17±0.00 ^f
Lignoceric acid (24:0)	0.11±0.00 ^b	0.15±0.01 ^{ab}	0.15±0.03 ^{ab}	0.14±0.001 ^{ab}	0.20±0.01 ^{ab}	0.19±0.05 ^{ab}	0.22±0.01 ^{ab}	0.12±0.00 ^{ab}	0.23±0.07 ^{ab}	0.32±0.07 ^{ab}	0.13±0.00 ^{ab}	0.15±0.03 ^{ab}
Percentage of oil	46.58±0.01 ^a	45.72±0.01 ^a	45.26±0.01 ^{ab}	38.20±0.10 ^{de}	44.86±0.15 ^{ab}	40.47±0.09 ^{cd}	30.52±0.47 ^f	37.33±0.90 ^e	38.31±0.21 ^{de}	39.25±0.15 ^{de}	43.07±0.40 ^{de}	44.19±0.1 ^{ab}

AI: IC-564585, B2: IC-564622, C3: IC-564631, D4: IC-564592, E5: IC-564630, F6: IC-564605, G7: IC-564624, H8: IC-564676, I9: IC-564660, J10: IC-564616. All the given values are means of three determinations±standard deviation. Means followed by different letters within a row are significantly different at $P < 0.05$ level

Table 3: Summary of unsaturated fatty acids, saturated fatty acids (%), and ratio of PUFA to SFA of linseed oils.

Fatty acids	BHU 1	BHU-B	A1	B2	C3	D4	E5	F6	G7	H8	I9	J10
Saturated fatty acids	12.61±0.00 ^h	12.68±0.01 ^h	13.48±0.05 ^h	13.74±0.00 ^f	19.99±0.01 ^a	14.19±0.05 ^e	19.29±0.04 ^b	14.95±0.00 ^e	14.45±0.05 ^d	14.75±0.07 ^e	13.71±0.00 ^f	14.32±0.02 ^{de}
Unsaturated fatty acids	86.66±0.01 ^a	86.57±0.00 ^a	85.80±0.04 ^b	85.58±0.00 ^{bc}	79.23±0.00 ^e	85.05±0.04 ^{cd}	79.97±0.02 ^f	84.32±0.00 ^e	84.78±0.06 ^{de}	84.45±0.22 ^{de}	85.61±0.00 ^b	84.67±0.30 ^{de}
MUFA	22.88±0.01 ⁱ	23.13±0.03 ⁱ	24.80±0.00 ^f	26.63±0.03 ^d	29.61±0.03 ^a	25.76±0.01 ^e	29.17±0.08 ^b	27.02±0.02 ^e	26.99±0.07 ^d	25.75±0.03 ^e	24.22±0.01 ^g	23.93±0.00 ^h
PUFA	63.78±0.02 ^a	63.44±0.03 ^a	61.00±0.04 ^c	58.95±0.02 ^{de}	49.62±0.03 ⁱ	59.29±0.03 ^e	50.80±0.10 ^b	57.29±0.02 ^g	57.79±0.00 ^f	58.70±0.19 ^e	61.39±0.01 ^b	61.05±0.00 ^{bc}
ω6	10.39±0.00 ^h	10.30±0.0 ^h	11.49±0.00 ^e	11.18±0.00 ^f	11.54±0.00 ^{de}	11.73±0.01 ^{cd}	10.90±0.09 ^h	11.60±0.00 ^{de}	12.10±0.00 ^b	11.26±0.07 ^f	11.85±0.00 ^e	12.70±0.00 ^a
ω3	53.39±0.02 ^a	53.14±0.03 ^a	49.51±0.04 ^b	47.77±0.02 ^d	38.07±0.02 ^h	47.56±0.02 ^d	39.60±0.29 ^f	45.69±0.02 ^e	45.69±0.00	47.44±0.12 ^d	49.53±0.00 ^b	48.35±0.00 ^e
PUFA: SFA	5.05±0.00 ^a	5.00±0.14 ^a	4.54±0.63 ^b	4.28±0.00 ^e	2.48±0.00 ^h	4.17±0.21 ^d	2.63±0.14 ^h	3.82±0.00 ^f	3.99±0.21 ^e	3.97±0.42 ^e	4.47±0.00 ^b	4.25±0.07 ^{cd}

A1: IC-564585, B2: IC-564622, C3: IC-564631, D4: IC-564592, E5: IC-564630, F6: IC-564605, G7: IC-564624, H8: IC-564676, I9: IC-564660, J10: IC-564616. All the given values are means of three determinations±standard deviation. Means followed by different letters within a row are significantly different at $P<0.05$ level

unsaturated fatty acids (MUFA) such as eicosenoic acid (20:1), oleic acid (18:1), and hexadecenoic acid (16:1) were determined to be in the scope of 22.88–29.61% [Table 3]. BHU-A, BHU-B, and IC-564660 accessions had the highest levels of ALA (53.39, 53.14, and 49.53%), respectively, and the lowest level of oleic acid (22.54, 22.66 and 23.87%), respectively [Figure 4]. These findings are similarly consistent with the previous studies showing raise in alpha-linolenic acid content in linseed oil resulted in a corresponding decrease in oleic acid content [22]. The accession IC-564631 had the lowest level of ALA and highest level of oleic acid content 38.7 and 28.93%, respectively, and these values were substantially different ($P < 0.05$) from other accessions. This might be due to a distinct linseed variety, origin and its accompanying environmental variance.

In the present study, PUFA: SFA ratio in linseed oil ranged from 2.48 to 5.05 showing that these oils contain a favorable fatty acid balance. The increased amount of unsaturated fatty acids in these accessions are significant because it aids in the reduction of total plasma cholesterol and low-density lipoprotein (LDL) concentrations by decreasing endogenous cholesterol production and reducing LDL particles [23]. In comparison to prior findings, the percentages of ALA in this analysis were higher than those obtained for linseed accessions cultivated in Egypt and America, which they reported 50% and 52%, respectively [6]. Whereas the higher percentage of ALA ranges from 47% to 59%, in Ethiopian linseed accessions were noticed by Wakjira *et al.* [21]. Choo *et al.* [22] reported 59.65% as well as 59%, respectively, in the linseed genotypes cultivated in Canada and New Zealand. Furthermore, Bozan and Tamelli [24] reported the ALA percentage level in flaxseed from Turkey as 56.5% to 61%, which is higher than our values.

Accessions evaluated in this study showed greater values of ALA (18:3), which is an important endogenous precursor for the synthesis of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), providing an advantage in the nutritional perspectives of intake of these seeds and seed oils. In general, ALA (18:3, ω^3) and linoleic acid (18:2, ω^6) are important fatty acids that have various functional aspects in the human diet and work together to control a number of physiological processes in humans [25]. Intake of omega-3 and omega-6 fatty acids has been correlated to a reduced rate of cardiovascular disease, hypertension, inflammation, asthma, arthritis, psoriasis, and various kinds of cancers [23,26].

3.2. Physico-chemical Properties

Out of 12 linseed accessions, five accessions BHU-A, BHU-B, IC-564585, IC 564616, and IC 564660 were selected based on their high content of ALA and highest genetic diversity [27] to carry out physico-chemical parameters such as SV, Iodine value, peroxide value, acid value, refractive index, and viscosity.

3.2.1. Saponification Value (SV)

Saponification number is an assessment of the relative molecular mass of fatty acids in an oil sample. High saponification number implies a larger percentage of lower molecular weight fatty acids of oils or *vice versa*. The saponification number obtained in different linseed oil samples are shown in Table 4. SVs were varied significantly ($P \leq 0.05$) within the oils of different accessions, the highest SV value was obtained in IC-564660 (193.5 mg KOH/g) followed by BHU-B (193.1 mg KOH/g) and the least SV value was obtained in BHU-A (192.1 mg KOH/g). These results are in comparison and consistent with reports of Popa *et al.* [28] and Herchi *et al.* [29] who obtained 190 and 198 mg KOH/g, respectively. The oil's high SV makes it ideal for use in the soap and cosmetics industries.

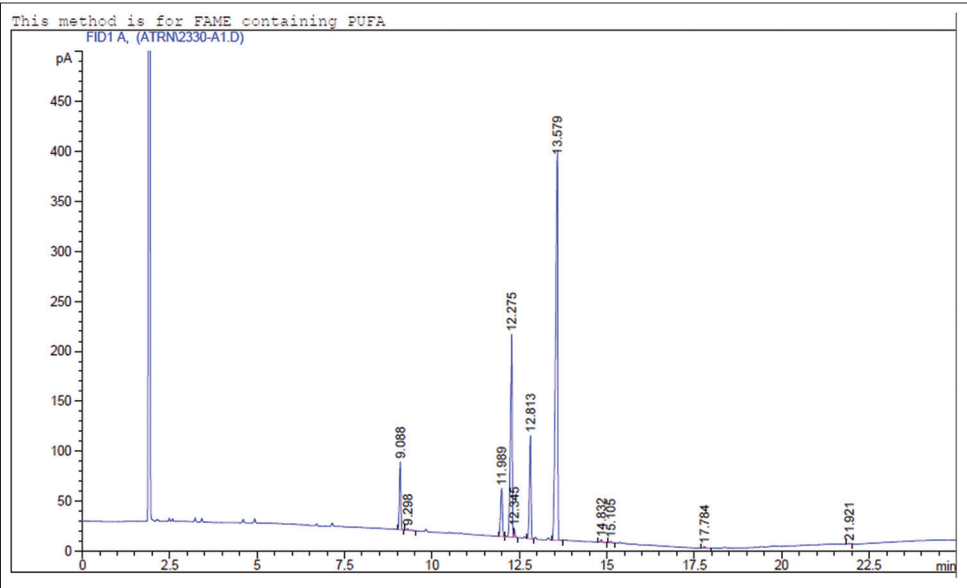


Figure 1: Gas chromatography-flame ionization detector chromatogram of the BHU-A linseed oil sample.

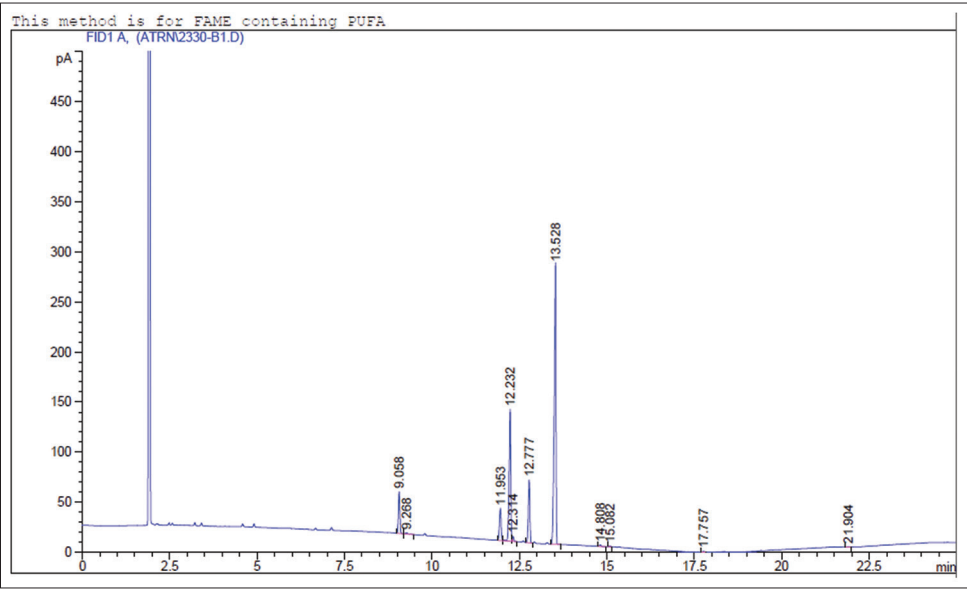


Figure 2: Gas chromatography-flame ionization detector chromatogram of the BHU-B linseed oil sample.

Table 4: Comparison of physico-chemical properties of linseed oil.

Characteristics	linseed oil samples				
	BHU-A	BHU-B	IC 564585	IC 564616	IC 564660
Saponification value (mg KOH/g)	192.16±0.15 ^c	193.12±0.10 ^{ab}	192.16±0.05 ^c	192.95±0.01 ^b	193.49±0.07 ^a
Iodine value (g Iodine/100 g)	178.83±0.05 ^a	173.39±0.4 ^c	176.83±0.05 ^c	177.21±0.05 ^b	175.30±0.01 ^d
Peroxide value (meq/Kg)	2.12±0.15 ^a	1.80±0.15 ^b	1.52±0.03 ^c	1.85±0.01 ^b	1.62±0.03 ^c
Acid value (mg KOH/g)	2.23±0.01 ^d	2.10±0.01 ^e	2.49±0.04 ^c	3.04±0.06 ^a	2.81±0.02 ^b
Refractive Index (RI) (at 40°C)	1.47±0.00 ^a	1.47±0.00 ^a	1.47±0.00 ^a	1.47±0.00 ^a	1.47±0.00 ^a
Viscosity at 40°C (cSt)	26.62±0.01 ^c	27.95±0.00 ^a	27.27±0.10 ^b	27.37±0.03 ^b	23.37±0.10 ^b

All the given values are means of three determinations±standard deviation. Means followed by different letters within a row are significantly different at *P*<0.05 level

3.2.2. Iodine value (IV)

The iodine index of fatty acids or vegetable oil determines the amount of unsaturation. It evaluates the durability of oils as well as permits

the overall unsaturation of the fatty acids to be measured subjectively. The statistical results showed that Iodine values were insignificantly (*P* ≤ 0.05) different among the five accessions. In the present

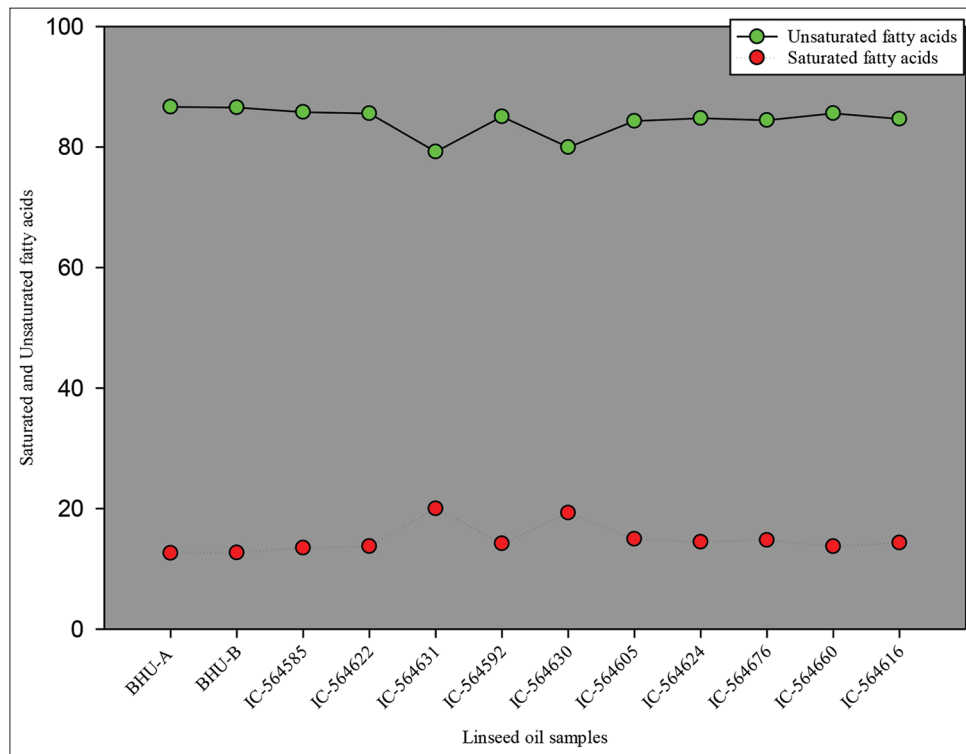


Figure 3: Percentage of saturated and unsaturated fatty acids in linseed oil samples.

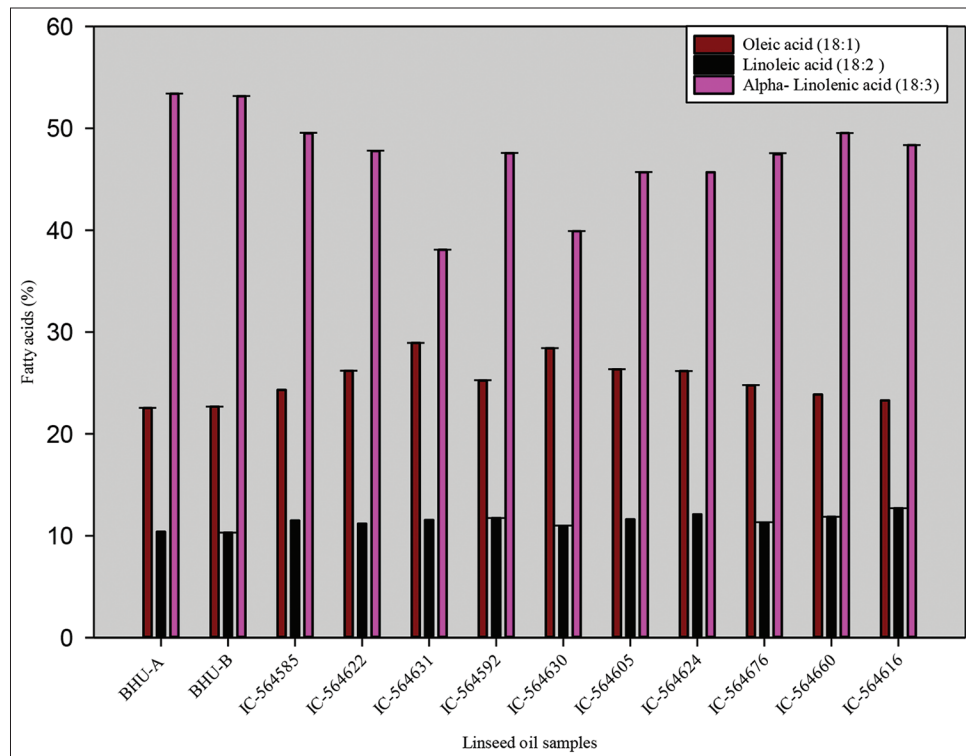


Figure 4: Percentage of oleic acid, linoleic acid, and alpha-linolenic acid in linseed oil samples.

investigation, it was examined that estimated iodine levels range from highest in BHU-A (178.8 g Iodine/100 g), IC-564616 (177.2 g Iodine/100 g) IC-564585 (176.8 g Iodine/100 g), IC-564660 (175.3 g Iodine/100 g), and least in BHU-B (173.4 g Iodine/100 g). These iodine values indicate that the oil consists of a significant level of

unsaturated fatty acids (86.66%) and a lower level of saturated fatty acids (12.73%). The iodine values obtained were smaller than those published by Zhang *et al.* [30], (195.03g/100g), and higher than those found by Herchi *et al.* [29] (160 g/100 g). The iodine content of flaxseed oil has been found to be in ranges from 180 to 203 g/100 g

of oil as reported by Przybylski [31]. According to Long *et al.* [32], the iodine value of flax seed oil is 162 g/100 g. Therefore, this oil may be regarded as a valuable asset for human nutrition, particularly due to its low amount of saturated fatty acids, which are linked with cardiovascular disease.

3.2.3. Peroxide value (PV)

The peroxide value is an estimation of the primary oxidation of products. According to the statistical data, the peroxide values of the five accessions differed insignificantly ($P < 0.05$). Highest Peroxide values obtained in BHU-A (2.12 meq/Kg), IC-564616 (1.85 meq/Kg), BHU-B (1.80 meq/Kg), IC-564660 (1.62 meq/Kg), and least in IC-564585 (1.52 meq/Kg), which were substantially below the peroxide value limit. According to Choo *et al.* [22], the peroxide value of cold-pressed flaxseed oil ranged from 0.5 to 2.9 meq/kg. The values obtained in this study did not exceed the maximum limit of 10–15 meq/kg of oil as for the Codex Alimentarius Commission [33].

3.2.4. Acid value (AV)

It is a measurement of the quantity of free fatty acids released from triglycerides as a result of hydrolytic breakdown (AOCS, 2009). It provides essential information on the edibility of oil and its appropriateness for industrial application. Acid values differed significantly ($P < 0.05$) across oils from different accessions. In the present study, the acid values for BHU-A (2.23 mg KOH/g), BHU-B (2.10 mg KOH/g), IC-564585 (2.49 mg KOH/g), IC-564616 (3.04 mg KOH/g), and IC-564660 (2.81 mg KOH/g) [Table 4]. These results were comparable and consistent with the results obtained in the previous studies [28,30]. Acid values were not greater than the recommended standards of (4.0 mg KOH/g) [33].

3.2.5. Refractive index (RI)

The refractive index represents the amount of unsaturation as well as the existence of unique compounds like hydroxyl groups. Refractive index values did not differ significantly ($P > 0.05$) among the accessions. The results indicated that the refractive index (40°C) values for BHU-A, BHU-B, IC-564585, IC-564616, and IC-564660 seed oils were 1.4722RI at 40°C, 1.4722RI at 40°C, 1.4722RI at 40°C, 1.4712RI at 40°C, and 1.4714 RI at 40°C, respectively. Our results are in agreement with earlier studies which reported the refractive index of flaxseed oil to be 1.475RI at 40°C [31], 1.469 RI at 40°C [28], and 1.479 RI at 40°C [30].

3.2.6. Viscosity

The viscosity of fluid indicates its internal resistance to flow and can be regarded as a measure of fluid friction [34]. Viscosity values differed significantly ($P < 0.05$) across oils from different accessions. The present results indicated that the Viscosity at 40°C (cSt) values for BHU-A, BHU-B, IC-564585, IC-564616, and IC-564660 seed oils were 26.62 at 40°C (cSt), 27.95 at 40°C (cSt), 27.27 at 40°C (cSt), 27.37 at 40°C (cSt), and 23.37 at 40°C (cSt), respectively.

4. CONCLUSION

The physico-chemical parameters and fatty acid profile of linseed (*Linum usitatissimum* L.) oil were analyzed in the present work. The fatty acid profile of the linseed oil samples indicates a significantly greater percentage of unsaturated fatty acids, with a predominance of ALA (53.39%) and linoleic acid (12.70%), as, well as beneficial index values, like PUFA: SFA, implying that these accessions are a rich resource of unsaturated fatty acids in human nutrition and this oil could be a good natural source of alpha-linolenic and linoleic acid.

various physico-chemical properties have been examined for five selected linseed oil samples and physico-chemical characteristics indicate good stability of the linseed oil. The outcomes of this comparative study can be beneficial for selecting economically and nutritionally significant linseed accessions. BHU-A and BHU-B accessions might be effectively employed for high content, good quality oil, and fatty acid composition.

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6. AUTHORS' CONTRIBUTIONS

BN: Carried out data analysis and interpretation, assisted in the experiment; MN, MS: Data analysis; TR, AT, Conducted the experiment; KK: Collected samples, designed the experiment, and draft manuscript preparation. The final manuscript was read and accepted by all authors.

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8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

10. DATA AVAILABILITY

The data generated and analyzed in this review article are included and the references are cited in the reference section.

11. PUBLISHER'S NOTE

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