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Evaluation of biochemical parameters in linseed (*Linum usitatissimum*)

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Abstract

The highest agricultural plant source of polyunsaturated fatty acids (PUFA), which are vital to human nutrition, is flax seeds, which have an oil content of around 36-40%. PUFA are quite prone to oxidation. As an Omega-6 fatty acid, linoleic acid makes up around 16 percent of the overall fatty acid content, whereas ALA makes up roughly 57%. Many experts have identified linseed as a modest double powerhouse in illness prevention due to its nutritional content. In present investigation, a total of ninety-two lines was evaluated for ten different biochemical parameters. Maximum protein percentage was found in genotype LMS-2015-81 followed by IC0585324 and SLS-140. The highest methionine content was investigated in genotype LMS-2015-22 tracked by IC0118861 and LMS-2015-42. While the highest proline amount was found in genotype IC049915 tracked by IC0498795 and IC0498843. The maximum phenol content was recorded in linseed line IC0096540 trailed by IC0498866 and LMS-2015-42. Maximum flavonoid content was contributed by genotype IC0448872 tracked by SLS-140. Maximum sugar content was recorded in genotype IC0967423 tracked by IC0448921 and IC0498538. Whereas, maximum antioxidant activity was evidenced in line IC0499156 tracked by IC0118861. While highest phytic content was recorded in linseed line IC0096672 tracked by IC0498866. Further these lines may be used to combine other traits in breeding programme to breed a high yielding cultivar along with nutrient rich.

Keywords: Antioxidant, flavonoids, linseed, methionine, phytic acid, protein

Introduction

The food and textile industries use flax, also known as linseed (*Linum usitatissimum*, family Linaceae), extensively as a crop plant for oil and fibre (Basch *et al.*, 2007) [8]. Due to the reported health benefits of omega-3 fatty acids and its remarkably high concentration of alpha linolenic acid (ALA), linseed is a profitable oilseed crop that is regaining popularity from its traditional use as a key ingredient in the production of oil (Schulze *et al.*, 2020) [47]. Consuming linseeds is beneficial to both human and animal health. Moreover, they contain a variety of bioactive phenolic components with biological activity that include antimicrobial, antioxidant, antiradical, and anticancer properties (Innes *et al.*, 2022) [27]. It is a major source of phenolic compounds known as lignans (>500 µg g⁻¹) and omega-3 fatty acids (ALA, which make up 52% of total fatty acids). When expressed on a moisture-free basis, the primary constituents of flaxseed are fat (41%), dietary fibre (28%) and protein (21%). The seeds taste pleasant and nutty and possess a crunchy and chewy texture (Yadav *et al.*, 2022) [56]. At a 9-10% water content, flaxseed can be stored for more than a year (Coskuner and Karababa 2007a) [16]. Many researchers have identified linseed as a minuscule two-fold force in disease prevention due to its nutritional profile. Since ancient times, there has been interest in the potential health benefits and disease prevention of linseed diets. Recently, there has been a resurgence of research on this topic.

Environmental factors and genotype have a major impact on the properties of seeds (Soto-Creda *et al.*, 2013) [53]. In general, phenolic compounds are found in the free state, conjugated with sugars or esters, or polymerized. They have an aromatic ring with one or more hydroxyl substituents (Shahidi, 2000) [49]. They are not equally distributed throughout plant tissues or cells, and they may be linked to cell wall constituents like proteins and polysaccharides.

Because lignans, phenols, tocopherols, and flavonoids all very significant phytochemicals are present in linseed, it may have the ability to protect lipids from oxidation.

The greatest source of PUFA, which are vital for human nutrition, among crop plants is linseed, which has an oil content of between 36 and 40% (Paliwal *et al.*, 2023) [40]. Given that it contains biologically active ingredients, such as linseed's prebiotic qualities and its positive effects on conditions like coronary heart disease, certain types of cancer, neurological, and hormonal disorders, it has consequently been regarded as the source of rising interest in nutrition and health research (Anonymous, 2010) [5]. Despite being a rich source of nutrients, it also contains anti-nutritional elements such as cyanogenic compounds (264-540 mg/100g) and trypsin inhibitors. These substances are harmful to people or they prevent protein from being available. To obtain the nutritional advantages of these seeds, processing them using thermal and mechanical methods such as roasting, microwave heating, autoclaving, and boiling is advised. The present investigation analyses the biochemical characteristics of linseed, including protein, methionine, proline, phenol, total flavonoids, total sugar, reducing and non-reducing sugars, antioxidant activity (DPPH), and phytic acid, which is an antinutritional molecule.

Materials and Methods

Plant material

A total of 92 genotypes of linseed acquired from AICRP on linseed, Crop Research Farm Mauranipur, BUA & T, Banda U.P., India. The experiment was conducted during Rabi season 2022-23 at Research Farm, Department of Genetics & Plant Breeding, College of Agriculture, RVSKVV, Gwalior, M.P., India. The seeds were sown in Augmented block design on 25th October 2022. The row to row and plant to plant spacing was kept 10 cm and 30 cm, respectively. The seeds were sown at the rate of 30-40 kg per ha. All agronomical practices were adopted to achieve a good crop. After seed maturity the plants were harvested and part of seeds were taken for biochemical analysis.

Protein extraction

Protein content was estimated by the method given by Lowry *et al.* (1951) [32]. Five hundred mg of plant materials were weighed and macerated in a pestle and mortar with 10 ml of 20 percent trichloroacetic acid. The homogenate was centrifuged for 15 min at 6000 rpm. The supernatant was discarded. To the pellet, 5 ml of 0.1 N NaOH was added and centrifuged for 5 min. The supernatant was saved and made up to 10 ml with 0.1 N NaOH. This extract was used for the estimation of the protein.

Methionine

Preparation of Samples for Assay- An amount of material equivalent to 1 gm. of protein was refluxed for 24 hours with 25ml of 20 percent hydrochloric acid. The hydrolysate was concentrated to a small volume in an air bath, and the residue was dissolved in water and boiled for a few minutes with a small quantity of norit. The hot mixture was filtered by suction, and the filtrate made up to a volume of 100ml with distilled water. For assays, 10 cc. aliquots of this solution were adjusted to pH 6.8 and diluted to 1 liter. One ml of the solution contained the protein equivalent of 100 y

percent. The solutions were stored under toluene in a refrigerator.

Preparation of Methionine Standards- The methionine standard solutions were prepared by dissolving 10 mg. of Z (-)-methionine in 1 liter of water. A 200ml aliquot of this solution was made up to 1 liter with distilled water. The two solutions containing 10 y and 2 y percent., respectively, were used for preparing the standard curve (Horn *et al.*, 1946) [25].

Proline extraction

Proline was extracted from the leaves using the method described by Bates *et al.* (1973) [9]. Briefly, 0.5 g of leaf sample was added into 10 ml of 3% sulfosalicylic acid and homogenized entirely at 3000 rpm for 10 min. afterwards, 2 ml of the filtered mixture was blended with 2 ml of ninhydrin followed by incorporation of 2 ml of acetic acid into each tube. The samples were then maintained in a water bath for 1 h and immediately placed on ice bath for a few min. Finally, the supernatants were subjected to absorption at 520 nm. The following equation is used to calculate the amount of proline in the extracts:

Proline in nmolmg⁻¹ FW or in µmolg⁻¹

$$FW = (Abs_{\text{extract}} - \text{blank}) / \text{slope} * Vol_{\text{extract}} / Vol_{\text{aliquot}} * 1 / FW$$

Where: Abs extract is the absorbance determined with the extract, blank (expressed as absorbance) and slope (expressed as absorbance nmol⁻¹) are determined by linear regression, Vol extract is the total volume of the extract, Vol aliquot is the volume used in the assay.

Total phenolics

Total phenolics were determined by the Folin-Ciocalteu method. Briefly, aliquots of 0.1 g lyophilized in flax seeds powder of samples were dissolved in 1 ml deionized water. This solution (0.1 ml) was mixed with 2.8 ml of deionized water, 2 ml of 2% sodium carbonate (Na₂CO₃), and 0.1 ml of 50% Folin-Ciocalteu reagent. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was read at 750 nm against a deionized water blank. Measurements were calibrated to a standard curve of prepared gallic acid solution, and the total phenolics concentration was expressed as mg gallic acid equivalents /100 g sample on a wet weight basis.

Total Flavonoids

Flavonoids content of different fractions was measured using a colorimetric assay developed by Pekkarinen S *et al.* (1999) [19]. A known volume (0.5 ml) of flavonoid extract or standard solutions of rutin (Sigma) was added to a 10 ml volumetric flask. Distilled water was added to make a volume of 5 ml. At zero-time, 0.3 ml of 5% (w/v) NaNO₂ was added to the flask. After 5 min, 0.6 ml of 10% (w/v) AlCl₃ was added and after 6 min, 2 ml of 1 M NaOH was added to the mixture followed by the addition of 2.1 ml distilled water. The absorbance was read at 510 nm and flavonoids content was expressed as mg rutin equivalents/100 g FW.

Reducing sugar

This method by Miller (1959) [35] has been widely used to

estimate the reducing sugar content of different samples. In our application, x mL standard or sample, 2 mL of DNS reagent and (2x) mL H₂O were added to a test tube, and the mixture was vortexed and incubated in a boiling water bath for 5 min. After cooling in ice bath, the absorbance of sample was recorded at 540 nm against a reagent blank. Linear regression equation was obtained using a glucose standard solution.

Total sugar

Total sugar is estimated as per method suggested by Morse (1947) [37] by preparing methanolic extract of plant sample and 1ml of plant extract was taken and about 4ml of anthrone reagent was added. After adding the reagent, the sample was kept in water bath at boiling point of water for eight minutes and then taken out the sample and let it cool to room temperature. Then 1.5ml of the sample was taken and the observation was recorded at 630nm absorption by.

Phytic acid

Harland & Oberleas (1977) [23] simplified the precipitation step and extracted phytic acid, about 0.2 g of dry, powdered sample was extracted 3 or 4 times with 3 ml 0.5 N HCl at 60° for the first few minutes and subsequent stirring at room temperature for 40 min. The combined extract was centrifuged and the supernatant adjusted to about pH 2. Phytic acid was precipitated with excess FeCl₃. The precipitate was dispersed in 0.5 ml water, treated with 0.5 ml 0.6 N NaOH for 30 to 50 min in a boiling-water bath and precipitate was estimated calorimetrically at 515 or 520 nm after reaction with hydroxylamine and o-phenanthroline.

Antioxidant activity (DPPH)

Following the method of radical scavenging activity was determined. The linseed powder (100 mg) was extracted in 2ml methanol. 1ml of supernatant was mixed with 3ml of 0.1mM DPPH and incubated for 30 min in dark. The absorbance was measured at 517nm and ascorbic acid was used as a positive control. DPPH radical scavenging activity was calculated using the equation:

$$\text{DPPH}\% = (\text{A}_{\text{blank}} - \text{A}_{\text{sample}}) / (\text{A}_{\text{blank}}) * 100$$

Where A_{blank} is the absorbance of the control reaction and A_{sample} is the absorbance of the sample.

Statistical analysis

The statistical analysis was performed by one-way ANOVA, followed by Dunnett's *t*-test. The results were expressed as the mean ± SE to show variations in a group. Differences were considered significant when *p* < 0.05.

Results and Discussion

Protein content

According to Singh *et al.* (2011) [52] and Chung *et al.* (2005) [15], lysin, tryptophane, glutamic acid, aspartic acid, methionine, and arginine are all limited by linseed protein. The data pertaining to protein content in linseed is provided in Table 1 and Fig.1. The protein content was recorded in range of 16.30% to 23.92%. Maximum protein percentage was found in genotype LMS-2015-81 (23.94%) followed by IC0585324(23.92%) and SLS-140 (22.79%). While minimum was found in genotype IC0521455 (16.30%) and IC0498489(16.90%). Similar findings were also reported by

Chung *et al.* (2005) [15], Gupta *et al.* (2021) [21], Kumar *et al.* (2021) [28] and Plaha *et al.* (2023) [44].

Methionine content

A precursor to homocysteine, cysteine, creatine, and carnitine, methionine is an aliphatic, sulphur-containing, essential amino acid. Recent studies have shown that methionine can control the innate immune system, digestive system, and metabolic functions in animals (Martinez *et al.*, 2017). The methionine content was investigated in range of 0.35 to 0.48 g/16g N (Table 1; Fig.1). Maximum methionine content was found in genotype LMS-2015-22 (0.48g/16g N) followed by IC0118861 (0.48 g/16g N) and LMS-2015-42(0.47 g/16g N). Whereas minimum was evidenced in genotype IC0498866 (0.35 g/16g N) tracked by IC0498486 (0.37g/16g N). These findings agreed with results of Chung *et al.* (2005) [15], Kumar *et al.* (2021) [28] and Plaha *et al.* (2023) [44].

Proline content

Significant functions of proline have been discovered in the developmental processes related to plant reproduction. Normal plant circumstances result in the largest concentrations of proline being found in the seeds and in the flowers, particularly in the pollen grains. Within the roots are the lowest layers. As an Osmo protectant, proline appears to be necessary for both the desiccation-dependent processes of embryogenesis and pollen formation. The maximum proline was found in genotype IC049915 (26.86 μ mol/g FW) followed by IC0498795 (26.84 mol/g FW) and IC0498843 (26.33 mol/g FW) (Table 1; Fig.1). While the minimum was evidenced in genotype IC0498724 (2.97 mol/g FW) and IC0342805 (3.20 mol/g FW). Similar results were also reported by Chung *et al.* (2005) [15], Singh *et al.* (2011) [52], Choudhary *et al.* (2021) [14], Kumar *et al.* (2021) [28] and Plaha *et al.* (2023) [44].

Total phenolic content

The cinnamic (C₆-C₃) and benzoic (C₆-C₁) acid derivatives make up the phenolic acid's family. They are distinguished by having a benzene ring replaced with one or more hydroxyl or methoxy groups and a carboxylic group (Larson, 1997) [29]. Naturally occurring hydrophilic antioxidants, phenolic acids may be found in a wide variety of foods, including fruits, vegetables, spices, and fragrant herbs (Silva *et al.*, 2000) [51]. The total phenolic content in linseed varied from 171 mg GAE/100 g to 350 mg GAE/100g with maximum in linseed line IC0096540 (350 mg GAE/100g) followed by IC0498866 (339 mg GAE/100 g) and LMS-2015-42 (337 mg GAE/100g). Whilst the minimum turned out to be in genotype IC0526118 (171 mg GAE/100mg) tracked by IC0385396 (171 mg GAE/100mg) (Table 1; Fig.2). Results were consistent with the findings of Chung *et al.* (2005) [15], Kumar *et al.* (2021) [28] and Pandey *et al.* (2023) [41].

Flavonoid content

Throughout the plant world, flavonoids are a class of naturally occurring benzo-γ-pyrane derivatives that have a variety of biological effects, including antioxidative activity (Pekkarinen *et al.*, 1999) [42]. According to Nieto *et al.* (1993) [39], flavonoids may have an antioxidant role in the lipid systems of food. The total flavonoid content varied from 14.02 mg/100 g to 20.79 mg/100g with highest in

genotype IC0448872 (20.89 mg/100 g) followed by SLS-140 (20.91 mg/100g) and IC0826162 (20.79 mg/100 g). While the minimum was recorded in genotype IC0498768 (14.02 mg/100g) and IC0346107 (14.09 mg/100 g) (Table1; Fig.2). These findings agreed with results of Chung *et al.* (2005) ^[15], Kumar *et al.* (2021) ^[28] and Plaha *et al.* (2023) ^[44].

Total sugar, reducing sugar and non-reducing sugar contents: The sugar content was found between 1.64% to 1.73% with maximum in genotype IC0967423 (1.76%) followed by IC0448921 (1.75%) and IC0498538 (1.73%) (Table1; Fig.3). Whilst minimum was noticed in genotype IC0526063 (1.64%) tracked by IC0356352 (1.64%). The non-reducing sugar content was recorded arrayed between 1.08 to 1.18 percent, with maximum in the genotype IC0498427 (1.18%) tracked by IC0526118 (1.18%). Whereas minimum was noticed in the genotype IC0526063 (1.08%) tracked by IC0526118 (1.08%). In respect to reducing sugar content, it was investigated between 0.55 to 0.57 percent with highest in the genotype IC0523801 (0.57%) tracked by LMS-2015-42 (0.57%) and IC0498392 (0.56%). These findings are consistent with the results of Chung *et al.* (2005) ^[15], Kumar *et al.* (2021) ^[28], Mishra *et al.* (2021) ^[36] and Plaha *et al.* (2023) ^[44].

Antioxidant activity

Antioxidants act as neutralizing agents by providing free

radicals with an electron and making them appear innocuous, to reduce the oxidative damage they cause to biological processes. Oxidative stress is largely linked to free radicals. The antioxidant activity ranged between 48.44% to 31.13% with maximum in line IC0499156(48.76%) followed by IC0118861 (48.44%). While minimum percentage reduction is seen in genotype IC0523801 (31.13%) tracked by IC0526118 (31.67%) (Table1; Fig.4). Analogous findings were reported by Chung *et al.* (2005) ^[15], Singh *et al.* (2011) ^[52] Kumar *et al.* (2021) ^[28] and Rathore *et al.* (2022) ^[46].

Phytic acid

Phytic acid has a chelating characteristic that causes it to attach to minerals and render them inaccessible. Iron, zinc, calcium, magnesium, and manganese absorption have all been shown to be inhibited by phytic acid (Bohn *et al.*, 2004; Phillippy, 2006) ^[11, 43]. Eliminating phytic acid improves the meal's nutritional value by increasing the bio-availability of several cations. Maximum phytic content was recorded in linseed line IC0096672 (0.14mg/g) followed by IC0498866 (0.14mg/g). While minimum in line IC0426926 (0.06mg/g) tracked by IC0564592 (0.06mg/g) (Table1; Fig.4). These findings are in accordance with the results of Chung *et al.* (2005) ^[15], Kumar *et al.* (2021) ^[28], Tomar *et al.* (2022) ^[55] and Rana *et al.* (2023) ^[45].

Table 1: Total content of protein, methionine, proline, phenol, flavonoids, sugar, antioxidant activity and phytic acid in linseed genotypes

S. No.	Genotypes	Protein (%)	Methionine (g/16 g N)	Proline (μ mol/g FW)	Phenol (mg GAE/100g)	Flavonoid (mg/100 g)	Total Sugar (%)	Reducing sugar (%)	Non-reducing sugar (%)	DPPH (%) inhibition	Phytic acid (mg/g)
1	IC0096672	19.27±0.199	0.357±0.01	25.027±0.13	314±0.12	19.303±0.32	1.737±0.022	0.563±0.011	1.173±0.037	46.73±0.071	0.068±0.022
2	IC0525976	17.927±0.04	0.38±0.006	20.637±0.08	270±0.16	16.233±0.33	1.707±0.024	0.563±0.013	1.143±0.035	47.97±0.071	0.238±0.026
3	IC0498660	20.17±0.021	0.457±0.01	3.907±0.050	224±0.15	17.393±0.31	1.747±0.022	0.563±0.011	1.183±0.034	47.97±0.061	0.218±0.021
4	IC0499165	17.4±0.1980	0.437±0.01	13.937±0.12	268±0.11	14.973±0.30	1.713±0.019	0.55±0.0091	1.163±0.036	35.99±0.069	0.128±0.024
5	IC0526058	23.363±0.17	0.427±0.01	10.047±0.07	257±0.15	18.113±0.32	1.687±0.025	0.563±0.011	1.123±0.036	37.88±0.066	0.138±0.023
6	IC0448872	20.533±0.05	0.463±0.00	12.127±0.12	209±0.13	20.887±0.27	1.737±0.024	0.553±0.009	1.183±0.027	41.42±0.078	0.178±0.019
7	IC0526063	17.257±0.03	0.43±0.012	9.637±0.080	306±0.11	15.19±0.330	1.637±0.025	0.553±0.012	1.083±0.032	34.21±0.065	0.128±0.020
8	IC0526118	21.92±0.047	0.443±0.02	7.557±0.110	171±0.11	17.143±0.27	1.657±0.019	0.573±0.012	1.083±0.031	46.25±0.073	0.058±0.021
9	IC0498486	17.393±0.08	0.367±0.01	24.067±0.05	230±0.12	20.283±0.24	1.697±0.020	0.553±0.011	1.143±0.037	31.67±0.058	0.178±0.020
10	IC305141	19.37±0.080	0.437±0.02	23.337±0.02	296±0.15	19.943±0.23	1.677±0.022	0.563±0.009	1.113±0.035	37.9±0.0760	0.058±0.019
11	IC0525920	20.637±0.17	0.46±0.020	10.277±0.05	277±0.15	18.297±0.27	1.657±0.021	0.573±0.011	1.083±0.038	44.92±0.062	0.168±0.021
12	IC0118855	17.42±0.115	0.477±0.09	8.307±0.110	231±0.15	16.577±0.25	1.717±0.023	0.553±0.011	1.163±0.032	40.88±0.077	0.128±0.024
13	IC0526166	23.253±0.25	0.46±0.025	19.767±0.12	249±0.13	14.773±0.23	1.697±0.021	0.563±0.011	1.133±0.028	35.93±0.076	0.078±0.020
14	IC0526087	22.113±0.39	0.443±0.09	6.277±0.130	200±0.14	15.657±0.26	1.647±0.024	0.563±0.011	1.083±0.035	39.92±0.078	0.148±0.022
15	IC0498866	21.667±0.32	0.347±0.03	22.977±0.10	339±0.15	18.03±0.240	1.647±0.024	0.553±0.012	1.093±0.034	37.87±0.068	0.058±0.020
16	IC0356352	18.62±0.081	0.407±0.09	7.697±0.070	323±0.14	16.017±0.28	1.637±0.022	0.553±0.013	1.083±0.032	46.68±0.061	0.238±0.025
17	IC0385396	21.687±0.17	0.397±0.01	5.197±0.080	171±0.13	14.977±0.29	1.667±0.024	0.573±0.013	1.093±0.028	35.95±0.079	0.198±0.022
18	IC0394130	17.64±0.114	0.463±0.05	25.917±0.04	202±0.15	19.48±0.310	1.687±0.019	0.573±0.011	1.113±0.035	42.59±0.067	0.118±0.024
19	IC0424547	19.427±0.09	0.423±0.01	20.397±0.10	289±0.16	19.387±0.30	1.737±0.025	0.553±0.013	1.183±0.037	37.61±0.065	0.068±0.019
20	IC0498538	21.147±0.09	0.46±0.017	23.197±0.06	250±0.13	18.153±0.26	1.747±0.021	0.563±0.011	1.183±0.033	46.72±0.071	0.158±0.021
21	IC0621689	19.417±0.14	0.47±0.006	26.077±0.06	180±0.15	16.203±0.27	1.677±0.019	0.563±0.009	1.113±0.034	40.91±0.059	0.158±0.027
22	IC0620658	23.54±0.075	0.377±0.00	21.787±0.15	207±0.16	15.02±0.290	1.647±0.024	0.563±0.012	1.083±0.039	46.16±0.065	0.118±0.027
23	IC0448921	18.497±0.07	0.39±0.006	18.877±0.08	179±0.14	19.043±0.32	1.747±0.023	0.563±0.012	1.183±0.039	42.93±0.078	0.128±0.023
24	IC0199753	23.247±0.59	0.48±0.021	15.967±0.14	235±0.11	18.80±0.290	1.727±0.024	0.573±0.012	1.153±0.036	35.1±0.0650	0.208±0.024
25	IC0413173	20.657±0.19	0.437±0.01	11.827±0.05	320±0.16	17.793±0.23	1.737±0.021	0.563±0.013	1.173±0.036	42.22±0.067	0.178±0.026
26	IC0053273	18.847±0.12	0.37±0.015	12.227±0.11	280±0.13	15.54±0.240	1.687±0.025	0.563±0.013	1.123±0.034	42.86±0.061	0.118±0.023
27	IC0096540	17.143±0.12	0.463±0.01	13.527±0.02	350±0.11	16.303±0.31	1.647±0.023	0.563±0.009	1.083±0.032	41.45±0.071	0.228±0.025
28	IC0526118	19.86±0.108	0.437±0.01	24.397±0.03	244±0.16	16.807±0.33	1.727±0.022	0.563±0.012	1.163±0.033	36.18±0.071	0.108±0.019
29	IC0054981	19.603±0.14	0.463±0.01	12.817±0.14	239±0.14	20.187±0.23	1.687±0.021	0.563±0.011	1.123±0.030	41.68±0.067	0.188±0.021
30	IC0585316	21.383±0.09	0.467±0.01	4.767±0.110	315±0.14	16.363±0.32	1.727±0.024	0.563±0.012	1.163±0.038	42.68±0.076	0.148±0.024
31	IC0342801	17.457±0.09	0.46±0.017	19.397±0.14	190±0.13	17.85±0.310	1.657±0.024	0.563±0.013	1.093±0.038	36.38±0.074	0.198±0.021
32	IC0449113	20.177±0.09	0.47±0.006	15.677±0.15	322±0.11	14.68±0.310	1.647±0.020	0.563±0.013	1.083±0.031	34.49±0.062	0.138±0.024
33	IC0499201	17.557±0.10	0.43±0.010	25.337±0.02	222±0.13	17.043±0.30	1.727±0.021	0.573±0.009	1.153±0.030	45.4±0.0660	0.198±0.020
34	IC0498517	17.47±0.065	0.43±0.012	18.597±0.09	203±0.13	17.323±0.31	1.737±0.020	0.563±0.011	1.173±0.032	36.58±0.065	0.108±0.027
35	IC0498689	19.697±0.16	0.47±0.012	19.207±0.15	281±0.15	18.743±0.23	1.737±0.020	0.553±0.012	1.183±0.038	38.8±0.0650	0.098±0.022
36	IC0564592	20.373±0.14	0.45±0.015	22.867±0.11	183±0.16	18.32±0.270	1.727±0.025	0.553±0.012	1.173±0.028	43.29±0.076	0.098±0.027

37	IC0498768	18.207±0.22	0.48±0.006	16.067±0.10	250±0.11	14.023±0.31	1.737±0.021	0.563±0.011	1.173±0.038	32.71±0.067	0.058±0.023
38	IC0096638	20.063±0.29	0.413±0.01	10.067±0.07	304±0.15	14.183±0.31	1.687±0.019	0.573±0.011	1.113±0.035	47.34±0.072	0.148±0.021
39	IC0498880	19.303±0.20	0.397±0.01	18.367±0.08	194±0.15	20.137±0.27	1.697±0.023	0.553±0.009	1.143±0.037	39.85±0.078	0.228±0.022
40	IC0498786	22.68±0.103	0.407±0.01	21.517±0.02	296±0.14	18.563±0.25	1.717±0.024	0.563±0.012	1.153±0.035	40.8±0.0660	0.068±0.023
41	IC0394118	21.333±0.17	0.38±0.006	4.787±0.120	250±0.13	16.237±0.27	1.737±0.024	0.563±0.013	1.173±0.037	44.51±0.069	0.228±0.021
42	IC0498989	21.297±0.17	0.377±0.01	20.327±0.02	300±0.15	19.413±0.24	1.667±0.024	0.573±0.011	1.093±0.031	32.25±0.059	0.238±0.026
43	IC0498843	22.633±0.08	0.46±0.012	26.327±0.08	172±0.14	16.877±0.27	1.717±0.022	0.553±0.011	1.163±0.032	38.71±0.065	0.168±0.023
44	IC0526514	17.767±0.05	0.477±0.01	12.367±0.04	337±0.14	16.61±0.280	1.667±0.019	0.573±0.011	1.093±0.031	43.2±0.0710	0.198±0.024
45	IC0521450	17.323±0.28	0.46±0.006	17.087±0.12	255±0.14	17.933±0.32	1.737±0.019	0.573±0.009	1.163±0.033	45.12±0.060	0.218±0.025
46	IC0521455	16.3±0.1200	0.43±0.015	13.847±0.13	238±0.11	14.33±0.260	1.687±0.025	0.573±0.013	1.113±0.033	32.96±0.074	0.238±0.027
47	IC0426926	21.1±0.1051	0.457±0.02	23.467±0.03	285±0.13	18.273±0.32	1.667±0.019	0.553±0.012	1.113±0.038	32.95±0.067	0.118±0.025
48	IC0498517	23.10±2.072	0.43±0.001	10.317±0.06	257±0.15	20.62±0.270	1.697±0.019	0.563±0.010	1.133±0.032	32.51±0.066	0.058±0.019
49	IC0342805	17.157±0.33	0.447±0.01	3.197±0.060	185±0.14	20.233±0.31	1.687±0.025	0.553±0.009	1.133±0.039	46.17±0.072	0.168±0.020
50	IC0342799	21.22±0.685	0.48±0.006	22.987±0.11	290±0.16	19.033±0.31	1.677±0.023	0.563±0.012	1.113±0.027	38.49±0.064	0.078±0.021
51	IC0498482	17.97±0.254	0.38±0.006	4.987±0.130	308±0.12	14.123±0.23	1.667±0.025	0.563±0.010	1.103±0.035	46.31±0.076	0.058±0.027
52	IC0585324	23.923±0.25	0.4±0.0060	22.287±0.06	240±0.11	17.097±0.23	1.667±0.022	0.573±0.009	1.093±0.037	39.97±0.070	0.098±0.027
53	IC0967423	17.593±0.21	0.45±0.012	23.537±0.07	333±0.16	15.68±0.290	1.757±0.025	0.573±0.009	1.093±0.039	44.92±0.075	0.138±0.025
54	EC0718843	18.723±0.19	0.457±0.00	6.457±0.110	225±0.14	16.263±0.25	1.717±0.020	0.553±0.011	1.163±0.030	45.14±0.069	0.108±0.023
55	IC0498489	16.897±0.39	0.46±0.017	4.357±0.070	243±0.11	16.10±0.230	1.677±0.019	0.553±0.013	1.123±0.030	38.34±0.075	0.118±0.022
56	IC0498561	19.0±0.5060	0.45±0.012	5.927±0.060	239±0.11	17.463±0.33	1.667±0.021	0.553±0.012	1.113±0.027	41.47±0.060	0.228±0.025
57	IC0499155	18.993±0.50	0.46±0.015	26.857±0.07	327±0.15	20.773±0.33	1.717±0.019	0.553±0.010	1.163±0.027	39.34±0.059	0.188±0.027
58	IC0499128	21.74±0.300	0.443±0.02	20.667±0.14	222±0.13	20.013±0.32	1.707±0.022	0.553±0.012	1.153±0.032	37.26±0.063	0.198±0.025
59	IC0572912	23.447±0.12	0.437±0.01	7.217±0.140	218±0.11	14.773±0.28	1.667±0.020	0.553±0.013	1.113±0.039	47.02±0.072	0.158±0.027
60	IC0499013	21.15±0.316	0.423±0.03	21.277±0.04	264±0.16	17.607±0.31	1.727±0.022	0.573±0.012	1.153±0.029	42.04±0.062	0.078±0.024
61	IC0599415	17.55±0.225	0.4±0.0060	11.397±0.09	171±0.14	14.913±0.27	1.697±0.021	0.553±0.012	1.143±0.036	43.57±0.070	0.098±0.024
62	IC0510935	17.24±0.164	0.433±0.01	16.997±0.09	335±0.11	19.563±0.30	1.717±0.022	0.563±0.009	1.153±0.030	39.75±0.073	0.108±0.020
63	IC0096678	23.87±0.320	0.44±0.006	24.587±0.08	216±0.15	14.35±0.330	1.647±0.020	0.563±0.009	1.083±0.029	47.06±0.075	0.158±0.023
64	IC0998770	18.803±0.49	0.38±0.006	23.107±0.10	225±0.16	18.457±0.32	1.657±0.021	0.573±0.012	1.083±0.027	31.74±0.065	0.078±0.024
65	IC0296039	18.25±0.509	0.457±0.01	24.647±0.07	288±0.11	16.57±0.290	1.727±0.022	0.563±0.013	1.163±0.038	32.35±0.075	0.148±0.025
66	IC0498605	21.357±0.24	0.37±0.015	9.847±0.020	281±0.14	14.95±0.310	1.697±0.020	0.573±0.010	1.123±0.035	32.14±0.060	0.078±0.026
67	EC0041621	20.217±0.62	0.437±0.01	26.157±0.11	330±0.13	16.96±0.320	1.667±0.023	0.553±0.011	1.113±0.029	44.59±0.070	0.128±0.022
68	IC0526162	22.117±0.18	0.37±0.006	9.347±0.150	179±0.12	20.793±0.24	1.717±0.025	0.553±0.009	1.163±0.027	38.97±0.072	0.168±0.019
69	IC0564677	17.233±0.52	0.387±0.01	11.967±0.12	200±0.13	20.297±0.25	1.717±0.025	0.573±0.009	1.143±0.035	41.92±0.078	0.228±0.020
70	IC0346107	17.813±0.37	0.473±0.01	25.457±0.09	203±0.13	14.087±0.25	1.717±0.019	0.563±0.010	1.153±0.032	36.23±0.077	0.158±0.024
71	IC0523801	18.817±0.41	0.437±0.01	23.497±0.03	324±0.11	15.97±0.230	1.717±0.024	0.573±0.009	1.143±0.037	45.05±0.069	0.178±0.027
72	IC0305053	18.213±0.29	0.433±0.01	12.447±0.12	206±0.15	17.883±0.33	1.697±0.021	0.563±0.011	1.133±0.036	31.13±0.076	0.238±0.026
73	IC0498392	17.143±0.38	0.463±0.01	9.917±0.100	267±0.12	18.983±0.25	1.727±0.024	0.573±0.012	1.153±0.038	36.74±0.067	0.128±0.020
74	IC0498938	17.02±0.216	0.46±0.006	10.067±0.06	311±0.11	16.863±0.25	1.707±0.020	0.553±0.009	1.153±0.030	32.53±0.064	0.188±0.027
75	IC0498795	19.773±0.27	0.48±0.006	26.837±0.03	187±0.15	18.423±0.30	1.707±0.019	0.553±0.010	1.153±0.029	45.12±0.059	0.168±0.022
76	IC0385383	18.823±0.33	0.447±0.01	19.567±0.04	334±0.12	15.103±0.27	1.637±0.025	0.553±0.013	1.083±0.038	44.37±0.059	0.238±0.024
77	IC0498427	23.24±0.406	0.453±0.01	6.907±0.100	184±0.12	17.597±0.23	1.737±0.022	0.553±0.012	1.183±0.034	46.3±0.0780	0.218±0.024
78	IC0259404	20.863±0.14	0.44±0.010	16.607±0.06	258±0.11	14.32±0.290	1.647±0.024	0.553±0.013	1.093±0.035	42.82±0.065	0.178±0.027
79	IC0526105	20.423±0.45	0.46±0.010	13.057±0.11	261±0.15	15.11±0.250	1.707±0.022	0.553±0.011	1.153±0.030	35.35±0.073	0.228±0.019
80	IC0498675	21.79±0.276	0.44±0.012	12.067±0.15	228±0.15	18.947±0.25	1.697±0.020	0.553±0.012	1.143±0.027	37.6±0.0800	0.158±0.026
81	IC0498724	22.927±0.23	0.387±0.01	2.967±0.100	247±0.16	18.057±0.26	1.717±0.024	0.553±0.011	1.163±0.033	37.5±0.0650	0.108±0.023
82	IC0498761	22.633±0.09	0.44±0.012	23.767±0.03	219±0.11	20.627±0.26	1.737±0.025	0.563±0.009	1.173±0.036	40.35±0.075	0.158±0.019
83	IC0424878	23.093±0.02	0.47±0.006	24.787±0.05	286±0.12	16.50±0.300	1.657±0.024	0.563±0.013	1.093±0.030	44.76±0.067	0.088±0.024
84	IC0499156	21.697±0.24	0.47±0.012	15.907±0.13	278±0.13	19.36±0.310	1.637±0.019	0.553±0.012	1.083±0.036	45.18±0.079	0.098±0.025
85	IC0356165	21.593±0.43	0.443±0.09	15.847±0.04	222±0.12	14.867±0.33	1.647±0.020	0.553±0.012	1.093±0.035	48.76±0.078	0.068±0.019
86	IC0118861	22.333±0.27	0.48±0.006	18.927±0.13	213±0.12	19.317±0.30	1.697±0.019	0.553±0.013	1.143±0.029	44.8±0.0620	0.088±0.019
87	LMS201581	23.86±0.170	0.447±0.09	23.517±0.03	303±0.12	17.297±0.32	1.727±0.024	0.563±0.009	1.163±0.039	48.44±0.073	0.208±0.021
88	LMS201542	22.797±0.54	0.43±0.012	20.907±0.12	337±0.12	18.047±0.24	1.697±0.022	0.573±0.011	1.123±0.038	46.08±0.075	0.238±0.021
89	LMS01420	22.177±0.09	0.447±0.01	5.577±0.120	245±0.15	14.203±0.28	1.637±0.019	0.553±0.009	1.083±0.038	40.79±0.074	0.158±0.021
90	LMS018-22	22.287±0.27	0.483±0.03	22.207±0.08	185±0.16	16.717±0.32	1.647±0.019	0.553±0.009	1.093±0.034	41.72±0.070	0.228±0.022
91	SLS-135	21.78±0.115	0.467±0.012	13.957±0.03	276±0.12	20.36±0.28	1.697±0.024	0.563±0.01	1.133±0.028	46.74±0.071	0.168±0.027
92	SLS-140	22.093±0.13	0.457±0.009	18.797±0.13	252±0.16	14.12±0.27	1.717±0.021	0.563±0.01	1.153±0.037	42.41±0.062	0.188±0.027
	SE(M)	0.336	0.013	0.279	0.15	0.23	0.019	0.013	0.035	0.061	0.025
	C.D _{0.05}	0.938	0.035	0.780	3.20	0.66	0.052	0.09	0.019	0.049	0.018

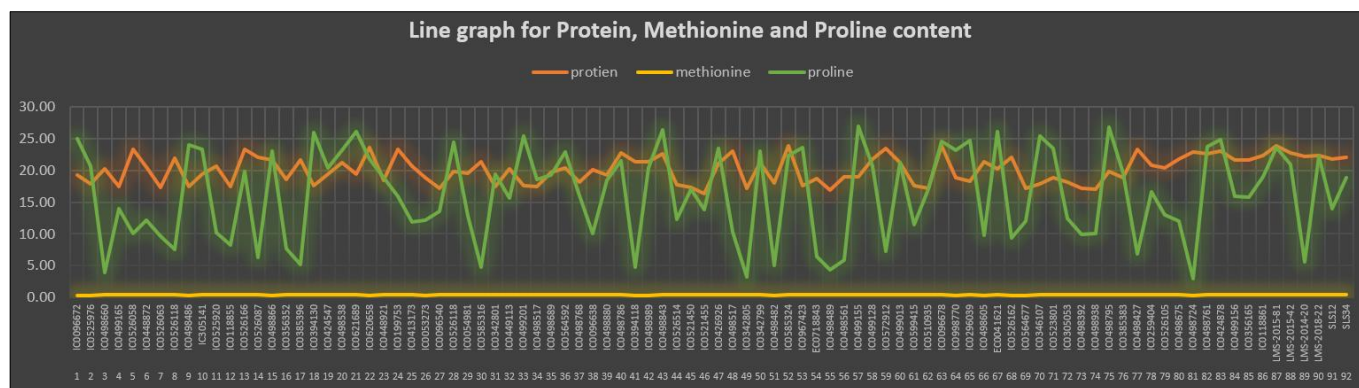


Fig 1: Line graph for protein, methionine, and proline contents

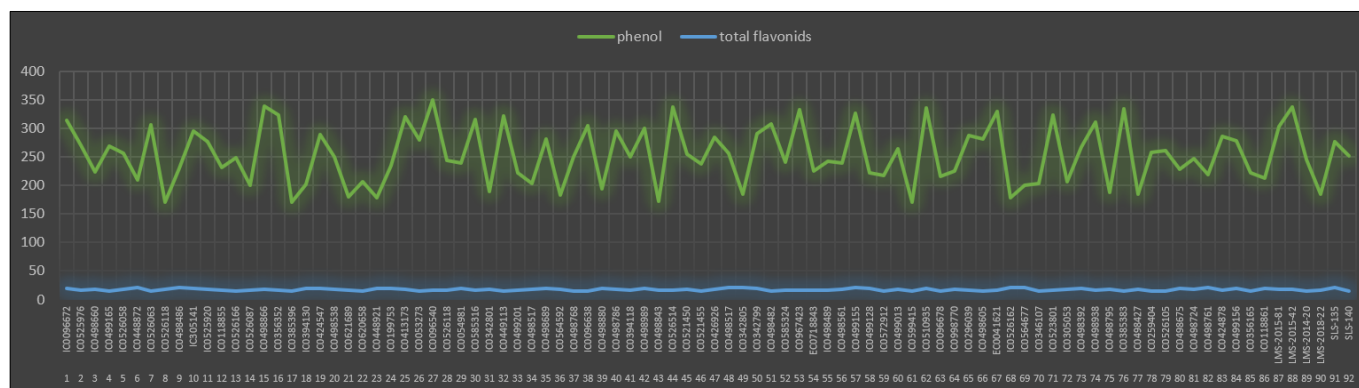


Fig 2: Line graph for phenol and total flavonoids

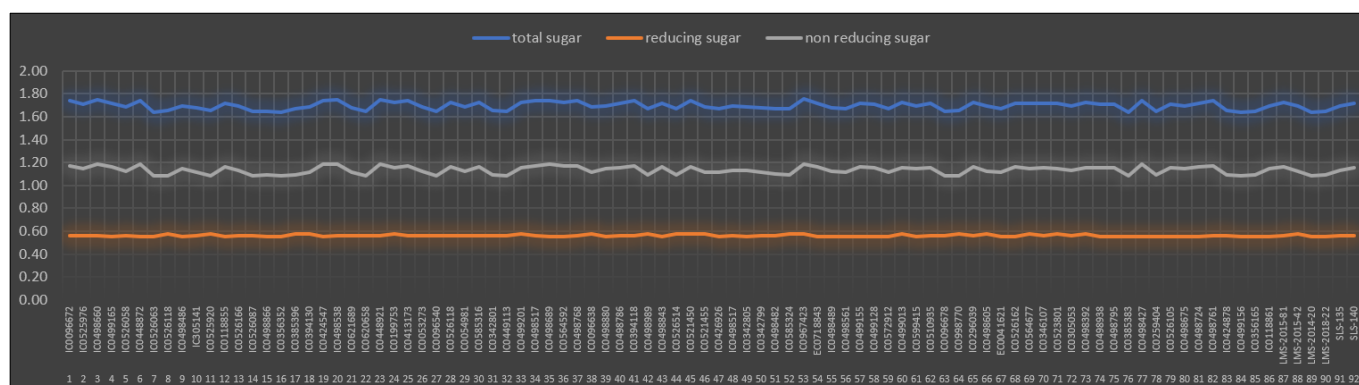


Fig 3: Line graph for total sugar, reducing sugar and non-reducing sugar

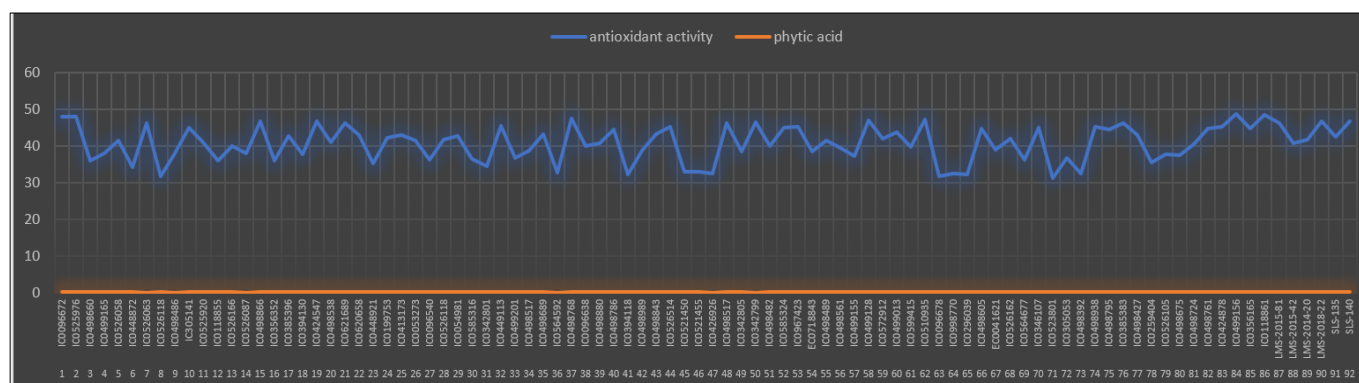


Fig 4: Line graph for antioxidant activity and phytic acid

Conclusion

This study analysing the biochemical parameters of linseed has provided valuable insights into nutritional composition and diverse biochemical profile constituting potential sources of rich antioxidants. The ninety-two genotypes were

evaluated for ten biochemical parameters which revealed that protein content in linseed ranged from 16 to 23 percent, Methionine content was found to be in between 0.38 to 0.45 g/16g N. Proline which is a stress indicator amino acid was found to be in moderate ranges which suggest that it can

tolerate enough stress during its growth period. Phenol plays an important role in induction of resistance in plants, they are involved in defence against pathogens, parasite, and predators, it ranged between 171 to 350 mg GAE/100g. Antioxidant activity was found superior to most of the oilseed crops thus further promoting its role for human consumption. Further research is warranted to explore the full range of health benefits associated with linseed consumption. Overall, the results of this experiment underscore the importance of incorporating linseed into a balanced diet to harness its nutritional benefits.

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