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# **Understanding the Power Packs: Flaxseed's Potential Benefits by Processing**

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Abstract: Flaxseed (Linum usitatissimum), a functional oilseed enriched with alpha-linolenic acid, lignans, dietary fiber, and protein, holds great potential as a nutraceutical ingredient. This study investigated the influence of germination, roasting, and fermentation on its nutritional composition, anti-nutritional factors, and functional attributes. Germinated flaxseeds (21.89  $\pm$  0.00 g/100 g) and roasted flaxseeds (26.56  $\pm$  0.43 g/100 g) showed higher protein contents compared to control seeds (19.7  $\pm$  1.85 g/100 g). Anti-nutritional compounds were reduced, with tannins decreasing to 2.95  $\pm$  2.12 mg GAE/100 g in germinated and 1.20  $\pm$  0.00 mg GAE/100 g in roasted flaxseeds, relative to 3.4  $\pm$  4.24 mg GAE/100 g in control. Similarly, phytic acid content declined from 46.8  $\pm$  0.00 mg/100 g in control to 34.0  $\pm$  0.00 mg/100 g and 2.4  $\pm$  0.00 mg/100 g in germinated and roasted seeds, respectively. Functional evaluation showed enhanced  $\alpha$ -amylase inhibitory activity, with germinated (85.47  $\pm$  0.75 U/L) and roasted seeds (86.01  $\pm$  1.52 U/L) performing better than control (80.1  $\pm$  0.76 U/L), indicating their potential in stabilizing blood glucose and reducing insulin resistance. Fermentation further improved digestibility and supported microbial activity, complementing the benefits of germination and roasting. The processing treatments demonstrated synergistic improvements, enhancing nutrient bioavailability, lowering anti-nutritional factors, and strengthening the functional potential of flaxseed. These findings highlight the relevance of simple, sustainable processing technologies in improving the nutraceutical and functional food applications of flaxseed.

Keywords: Flaxseed, Germination, Roasting, Fermentation, Anti-Nutritional Compound.

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#### I. INTRODUCTION

Flaxseeds belonging to the family Linaceae, Linum usitatissimum- commonly known as flaxseed or linseed is a nutrient-rich crop valued for both its culinary and functional properties (Kaur et al., 2018). This annual herb bears delicate blue flowers and produces small, smooth, glossy seeds that are flat and oval in shape, varying in color from golden yellow to reddish brown. They possess a pleasant nutty flavor with a texture that is both crisp and slightly chewy (Gutte et al., 2015). Among the various types, brown flaxseeds are the most widely consumed. Nutritionally, flaxseeds are notable for their oil rich in alpha-linolenic acid (ALA), substantial protein content, essential minerals, and lignan-rich dietary fiber. They are also a valuable source of bioactive compounds such as secoisolariciresinol diglucoside (SDG), lignans, and omega-3 fatty acids. On average, flaxseeds contain approximately 55% ALA, 28-30% protein, and about 35% fiber, while their carbohydrate content is minimal, around 1 g

per 100 g (Goyal et al., 2014). They are among the richest plant-based sources of ALA and lignans, which act as phytoestrogens. Compositionally, flaxseeds are complex, providing oil, protein, soluble polysaccharides, phenolic compounds, vitamins (A. C. E. and F), and minerals such as phosphorus, magnesium, potassium, sodium, iron, copper, manganese, and zinc (Goyal et al., 2014). For individuals following vegetarian diets, flaxseed oil can serve as an alternative to fish oil and may aid in improving insulin sensitivity in those with diabetes or prediabetes. Evidence also suggests that flaxseed consumption could lower the risk of both type 1 and type 2 diabetes, as omega-3 and omega-6 fatty acids influence insulin function by modifying phospholipid membrane composition (Bhardwaj et al., 2015). Hence, the present study focuses on characterizing processed flaxseeds through assessing its nutritional composition, antinutritional constituents, functional components and αamylase inhibitory potential.

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#### II. MATERIALS

The flaxseeds were collected from D-mart at Mysore road, Bengaluru. After collection, the flaxseeds were

germinated and grounded with conducting proximate analysis as shown in flow chart no 1 at the department of food and nutrition at Padmashree Institute of Management and Sciences, Bengaluru.

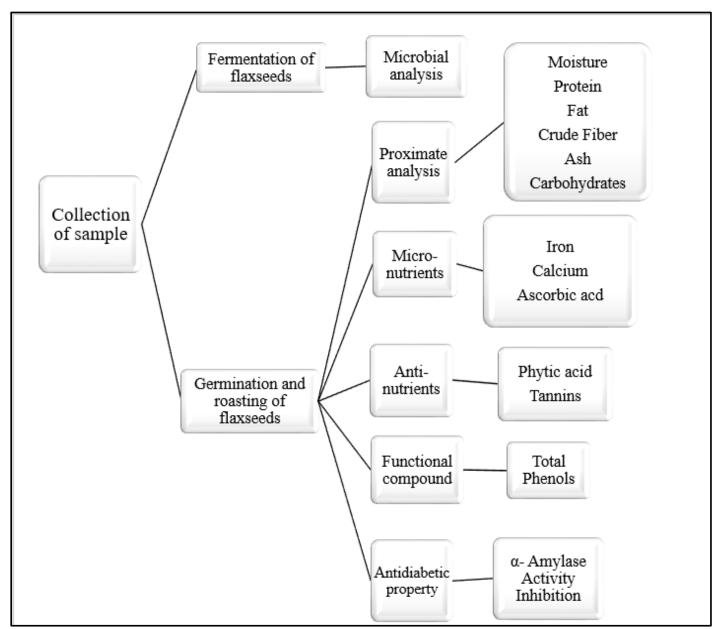


Fig 1 Nutritional Analysis of Processed Flaxseed

# III. METHODS

#### A. Germination of Flaxseeds

50g of flaxseeds were washed before keeping for germination and they were germinated for 7 days under refrigerated condition at 5°C. For every 2 days flaxseeds were sprinkled with water and after the germination, they were sun dried and grounded for further analysis.

# B. Roasting of Flaxseeds

The flaxseeds were roasted by dry roasting them for 4 minutes and they were grinded into a fine powder using a mixer grinder.

# C. Fermentation of Flaxseeds

20g of flaxseeds were washed and soaked in buttermilk (5g of curd and 1g of crystal salt in 75 ml of water) for 12 hours at 23°C. After the fermentation, the seeds were dried in hot air oven for 4 hours at 100°C. After drying, they were grounded for microbial analysis.

# D. Microbial Analysis of Fermented Flaxseeds

1g of grounded fermented flaxseeds were serially diluted (10<sup>-1</sup>, 10<sup>-2</sup>, 10<sup>-3</sup>, 10<sup>-4</sup>, 10<sup>-5</sup>, 10<sup>-6</sup>), and inoculated in de Man Rogosa Sharpe (MRS- selective media for lactic acid bacteria) agar. The petri plates were placed in incubator for 24 hours at 36°C. The bacterial growth was observed and

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Gram's Staining was performed to determine the Lactic acid bacteria under compound microscope.

# E. Determination of Moisture Content by Evaporation Method

Each sample was dried in an oven maintained at 100°C, with mass measurements recorded at half-hour intervals until no further weight loss occurred. (AOAC 930.04).

#### F. Estimation of Protein by Kjeldhal Method

Flaxseed samples (0.1 g) are digested using 10 ml of concentrated sulphuric acid and 3 g of a catalytic mixture (copper sulfate and sodium sulfate in a 1:5 ratio). After digestion, sodium hydroxide (40%) is added to make the solution alkaline, converting ammonium sulfate to ammonia gas, which is distilled and captured in a receiving flask containing 4% boric acid with a mixed indicator. The ammonia reacts with boric acid to form ammonium borate. The nitrogen content is then determined by titrating the ammonium borate with 0.1 N hydrochloric acid. (AOAC 978.04).

#### G. Estimation of Fat by Soxhlet Method

For Soxhlet extraction, the pulverized dried sample is loaded into a permeable extraction thimble and positioned within the extraction apparatus located between the solvent reservoir (commonly ether) and the condensing unit. After extraction, the solvent is evaporated, and the remaining lipid mass is measured (AOAC 930.09).

## H. Estimation of Crude Fiber

Defatted samples were treated with 200 mL sulphuric acid for 30 minutes, then washed with boiling water until neutral. They were then treated with 200 mL sodium hydroxide for 30 minutes, washed again, and the residue transferred to a pre-weighed dish, dried at  $130\pm2^{\circ}\text{C}$  for 2 hours, cooled, and weighed. The residue was then ignited at  $600\pm1$  5°C for 30 minutes, cooled in a desiccator, and reweighed (AOAC 930.10).

# I. Determination of Ash Content

Ash content determination involved weighing the flaxseed samples before and after the incineration. The results can be expressed either on a moisture-free or as-received basis depending on the analytical requirements. The sample was held at 500-600°C for 3 hours (AOAC 930.09).

#### J. Determination of Carbohydrate Content by Difference Method

Carbohydrates in flaxseed were quantified by calculating the difference between total components and the sum of moisture, protein, fat, and ash contents. (Wijaya and Romulo, 2021).

# K. Determination of Energy

Energy was measured using Atwater system method. The energy content was estimated using a standard analytical approach. (Food energy- methods of analysis and conversion factors: 2003).

#### L. Estimation of Amylase Activity Inhibition

For assessing amylase inhibition, 1 mL of flaxseed extract was mixed with  $\alpha$ -amylase (1 U/mL) and allowed to pre-incubate for 30 minutes. Then, 1 mL of 1% starch solution was added and incubated at 37°C for 10 minutes. The reaction was stopped by adding 1 mL DNS reagent and heating in a boiling water bath for 5 minutes. Blanks were prepared without the extract and without the enzyme, using buffer instead. Absorbance was measured at 540 nm to assess enzyme activity. The  $\alpha$ -amylase inhibition was measured and expressed in units per liter (U/L) of enzyme activity inhibited. (Bhutkar and Bhise, 2012).

#### M. Estimation of Phytic Acid by using Wade Reagent

Phytate content was estimated by extracting 0.5 g of sample with 2.4% HCl, shaking for 16 hours, and centrifuging. The supernatant was treated with NaCl, recentrifuged, and diluted to prepare a working standard. This was reacted with Wade reagent, and phytate content was calculated using a standard curve (64–320  $\mu$ g/mL) by measuring absorbance at 500 nm. (Gai *et al.*, 2007.)

# N. Estimation of Total Phenols by Folin-Ciocalteu Method

The phenolic compound concentration in the flaxseed extract was determined through the Folin-Ciocalteu colorimetric method, utilizing gallic acid as the calibration standard for quantification purposes. Varying volumes of extract and water were mixed, followed by the addition of 5 mL of 7% sodium carbonate and incubation for 20 minutes, then 0.5 mL Folin-Ciocalteu reagent was added and incubated for 10 minutes. Absorbance was measured at 660 nm, and results were expressed as gallic acid equivalents. (Tambe and Bhambar, 2014)

#### O. Estimation of Tannins by Folin-Ciocalteu Method

Total tannin content in flaxseed extract was estimated using the Folin-Ciocalteu method with gallic acid as the standard. Various volumes of the extract and distilled water were combined, followed by the addition of 1 mL of 35% sodium carbonate, and the mixture was incubated for 20 minutes. Afterward, 0.5 mL of Folin-Ciocalteu reagent was introduced and the solution was incubated for an additional 10 minutes. The absorbance was then measured at 660 nm, and the tannin content was reported as gallic acid equivalents. (Tambe and Bhambar, 2014)

#### P. Determination of Ascorbic Acid by Iodometric Method

To estimate ascorbic acid, 10 mL of standard solution (125 mg in 50 mL water) was titrated with iodine using 1% starch as an indicator until a blue color appeared. The volume of iodine used was recorded. The same procedure was followed for 10 mL flaxseed extract (150 mg of germinated or normal flaxseed in 50 mL water). The ascorbic acid content was calculated based on the titration volume. (Bhavya *et al.*, 2023).

#### Q. Estimation of Iron by using Ammonium Thiocyanate

Iron content was estimated using the ammonium thiocyanate colorimetric method. Ferric ions in the sample were treated with nitric acid and ammonium thiocyanate to form a red ferric thiocyanate complex. Iron content was

quantified by matching sample readings to a standard calibration curve after measuring their absorbance at 480 nm with a UV visible spectrophotometer. (Goswami and Kalita, 1988)

#### R. Estimation of Calcium by using EDTA

1ml of flaxseed extract was placed in a conical flask, mixed with 4ml of 2 M sodium hydroxide, and then diluted to volume with 25ml of distilled water. After adding 2–3 drops of Eriochrome Black-T indicator, the solution was titrated with 0.01M EDTA until the colour changed from pink to blue. The quantity of EDTA consumed was noted for mineral concentration analysis. (Bird *et al.*, 1961).

#### S. Statistical Analysis

The data were reported as means  $\pm$  standard deviation (SD) and statistical analysis primarily utilized graphs and calculations, using Microsoft Excel employed for tabulation. A Student's t-test was conducted to determine P-value to check its significance (\*P $\geq$ 0.05). The results were presented in tables, accompanied by discussion.

#### IV. RESULT AND DISCUSSION

#### > Processing treatments

Flaxseeds were germinated under cold condition (5°C) to prevent the growth of molds that were appearing during the

germination process. Germination lies in the activation of metabolic pathways triggered when seeds absorb water, leading to enzyme synthesis and mobilization of stored reserves (Bewley, 1997). Germination of flaxseeds enhances their nutritional quality by significantly increasing protein, essential amino acids, vitamins, and antioxidant compounds such as phenolics, flavonoids, and lignans, while reducing anti-nutritional factors like phytic acid and cyanogenic glycosides (Wang *et al.*, 2018).

Flaxseeds were roasting on a low flame heat to prevent nutrient loss and retain antioxidant capacity. Roasting flaxseeds induces Maillard reactions and thermal degradation of cellular structures, which release bound phenolics and lignans, thereby increasing measurable antioxidant compounds and improving oil oxidative stability (Waszkowiak *et al.*, 2020).

The flaxseeds were fermented using butter milk which naturally contains *Lactobacillus* species and Gram's staining was done and the seeds were found to contain Gram positive bacteria by observing the colour (purple) of the bacteria under microscope. Fermentation of flaxseed polysaccharides by bacteria generates bioactive metabolites that can suppress glucose absorption and fat cell formation, indicating a possible pathway for supporting metabolic regulation and weight control. (Lin *et al.*, 2021)



Fig 2 a- Germinated flaxseeds; b- Roasted flaxseeds; c- Fermented flaxseeds; d- Microbial analysis of fermented flaxseeds

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#### > Effect of processing on nutritional composition

The results of chemical composition of normal (CFS), germinated (GFS), roasted flaxseeds (RFS) are depicted in table-1, wherein GFS and RFS had higher protein  $(21.89\pm0.00 \text{ g}/100\text{g})$  and  $26.56\pm0.43 \text{ g}/100\text{g})$  when compared to CFS (19.7±1.85 g/100g). The changes in nutrition composition were also observed in Kajla et al., 2017, where protein in germinated flaxseed was higher (22.51±0.57 g/100g) compared to normal flaxseed (21.50±0.42 g/100g) and also in the study by Labban, 2022, observed that the protein content increased in roasted flaxseeds (15.12±0.25 when compared with unroasted flaxseeds g/100g) (14.85±0.30). The crude fat content was relatively lower in germinated flaxseeds (35.12±7.6 g/100g) but significantly higher in the roasted flaxseeds (38.47±0.53 g/100g) when compared with the control seeds (38.27±2.22 g/100g) which had the significant values (P=0.72 and 0.46). Similar results were seen in Kajla, et al., 2017, where there was significant decrease in the fat content of germinated flaxseed (42.56±0.35 g/100g) compared to normal flaxseed (44.82±0.07 g/100g), this might be because of increased activities of lipolytic enzyme during germination which hydrolyses fats into free fatty acids and glycerols and also by Prajapati et al., 2016, the fat content in roasted flaxseeds increased compared to normal flaxseeds (40.24±0.44 to 43.86±0.37/100g). Crude fiber content in control flaxseeds (21.82±2.29 g/100g) was higher than that of germinated flaxseeds (20.8±0.34 g/100g) and roasted flaxseeds (19.22±0.34 g/100g) which were found to be significant (P= 0.60 and 0.15). The changes in crude fiber was also observed in Prajapati et al., 2016, where the study shows nonsignificant decrease in unroasted & roasted flaxseed  $(5.37\pm0.10 \text{ to } 4.10\pm0.04 \text{ g}/100\text{g})$ . The moisture content of the control flaxseed (7.04±0.16%) was higher than germinated flaxseeds  $(4.56\%\pm0.00)$  and roasted flaxseeds  $(0.09\pm0.13\%)$ . The carbohydrate content in germinated flaxseed (15.7±7.94 g/100g) and roasted flaxseeds (12.21±0.30 g/100g) was higher when compared to that of the control flaxseeds  $(4.56\pm2.07 \text{ g/}100\text{g})$  with significant values (P= 0.32 and 0.35). The ash content of germinated flaxseed (1.87±0.00 g/100g) and roasted flaxseeds (3.45 g/100g±0.21) was slightly higher than the control flaxseed (1.78±0.12 g/100g) and was significant only for germinated flaxseeds (P= 0.25). The energy in the germinated flaxseed was slightly lower (466.48±36.62 kcal/100g) and relatively higher in roasted flaxseeds (501.37±1.8 kcal/100g) than the control flaxseed (468.77±19.16 kcal/100g) which was significant for both germinated and roasted flaxseeds (P= 0.48 and 0.13). The increase in the energy between roasted (599.49±11.11 kcal/100g) and normal flaxseeds (566.27±3.48 kcal/100g) was also found in Prajapati et al., 2016. There were no changes observed in the ascorbic acid on germinating (0.023 g/100g±0.00) compared to control flaxseed (0.023 g/100g±0.00), but the impact was observed on roasting the flaxseeds (0.011±0.00 g/100g). The iron content of germinated flaxseeds (17.5 $\pm$ 3.53 mg/100g) and the roasted flaxseeds (15.3 $\pm$ 1.8 mg/100g) was relatively higher than control flaxseeds (12.15 $\pm$ 1.76 mg/100g). Roasting was found to enhance the iron content of flaxseeds (6.03 $\pm$ 0.04 to 6.17 $\pm$ 0.08 mg/100g), as observed by Prajapati *et al.*, 2016. The calcium was determined, where germinated flaxseed (800 $\pm$ 0.00 mg/100g) was significantly (P= 0.19) higher and lower in the roasted flaxseeds (460  $\pm$ 28.28 mg/100g) when compared with the control flaxseed (600 mg/100g $\pm$ 0.00).

#### ➤ Effect of Processing on Functional Components

The functional component such as phenol contents was found to be significantly (P= 0.14) higher in germinated flaxseed (8.25  $\pm 17.67$  mg GAE/100g) and lower in the roasted flaxseeds (1.8±0.00 mg GAE/100g) than the control flaxseeds (7.25±10.60 mg GAE/100g). Similar increase was reported by Vrancheva et al., 2019, where the germinated flaxseed (2.45±0.02 mg GAE/g DW) was higher compared to raw flaxseed (0.29±0.00 mg GAE/g DW) and similarly there was decrease in the phenolic content of roasted flaxseed (432.23 mg GAE/100g) compared to raw flaxseed (480.15 mg GAE/100g), which was seen in Yadav et al., 2020, which might be because of thermal degradation that causes changes in molecular structure. Flaxseeds are abundant in phenolic compounds, including lignans and phenolic acids, which exhibit potent antioxidant and anti-inflammatory properties, aiding in the reduction of oxidative stress and neutralization of harmful free radicals in the body. (Huang et al., 2024)

#### ➤ Effect of Processing on Anti-Nutrients

The tannin content had relative drop in both germinated flaxseeds (2.95±2.12 mg GAE/100g) and roasted flaxseeds (1.20±0.00 mg GAE/100g) when compared to control flaxseeds (3.4±4.24 mg GAE/100g) which was significant for germinated flaxseeds (P= 0.1). While, the phytic acid also found to be decreased in both germinated flaxseeds (34.0±0.00 mg/100g) and roasted flaxseeds (2.4±0.00 mg/100g) when compared to control flaxseeds (46.8±0.00 mg/100g). Similar results were recorded by Kajla et al., 2017, where the phytic acid decreased in germinated flaxseed (11.6 g/kg) compared to raw flaxseeds (23.6 g/kg). During the germination process, the activation of phytase enzymes may lead to a decline in phytic acid content (Kajla et al., 2017). The results were similar in case of Pant and Awasthi, 2015, where the phytic acid decreased in roasted flaxseeds (1.51g/ 100g) compared to raw flaxseed (2.03 g/100g). Simultaneously, the tannin contents also decreased in roasted flaxseeds (7.05 mg/100g) compared to raw flaxseeds (7.26 mg/100g). The reduction of anti-nutritional factors, such as phytic acid and tannins, through techniques such as thermal processing, fermentation, or enzymatic modification, can improve the absorption of vital minerals like calcium, magnesium, zinc, and iron by increasing their bioavailability (Dulinski et al., 2017).

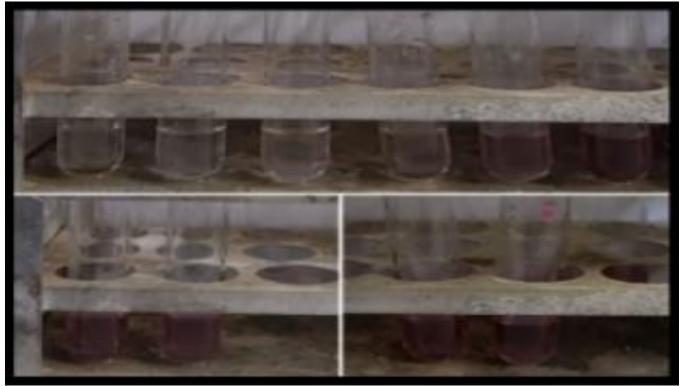


Fig 3 Estimation of Phytic Acid

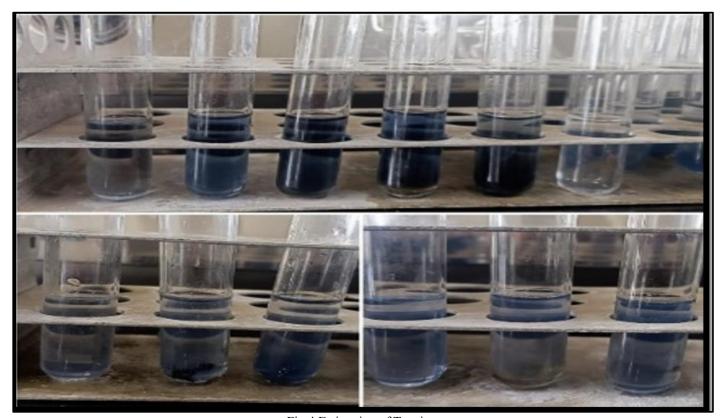


Fig 4 Estimation of Tannins

# ➤ Effect of Processing on Amylase Activity Inhibition

The  $\alpha$ -amylase activity inhibition of geminated, roasted and control flaxseed were determined and the results were obtained as shown in Table 4. The  $\alpha$ -amylase activity was highly inhibited in germinated flaxseeds (85.47±0.75 U/L) and roasted flaxseeds (86.01±1.52 U/L) when compared

to control flaxseeds ( $80.1\pm0.76$  U/L). By inhibiting this enzymatic action, bioactive constituents of flaxseeds can aid in stabilizing blood glucose levels and lowering the incidence of insulin resistance, thereby serving as potential nutraceutical agents for supporting metabolic health. (Hano *et al.*, 2013)

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Fig 5 Estimation of Amylase Activity Inhibition

Table 1 Nutritional Composition of the Flaxseeds

	Tuote i i tui ilionai eoin	Septiment of the Flambeeds	
Nutrient	CFS* (g/100g)	GFS* (g/100g)	RFS*(g/100g)
Energy (kcal)	468.77±19.16	466.48±36.62*	501.37±1.8*
Moisture (%)	7.04±0.16	4.56±0.00	$0.09\pm0.13$
Carbohydrates (g)	4.56±2.07	15.7±7.94*	12.21±0.30*
Protein (g)	19.7±1.85	21.89±0.00*	26.56±0.43
Fat (g)	38.27±2.22	35.12±7.6*	38.47±0.53*
Crude Fiber (g)	21.82±2.29	20.8±0.34*	19.22±0.34*
Ash (g)	1.78±0.12	1.87±0.00*	$3.45 \pm 0.21$
Ascorbic acid (g)	0.023±0.00	$0.023\pm0.00$	0.011±0.00
Iron (mg)	12.15±1.76	17.5±3.53	15.3±1.8
Calcium (mg)	600±0.00	800±0.00*	460±28.28

(n=2); \*GFS- Germinated Flaxseeds; \*CFS- Control Flaxseeds; \*RFS- Roasted Flaxseeds; \*P ≥ 0.05 (significance)

Table 2 Functional Compound of the Flaxseeds

Functional compound	CFS* (mg/100g)	GFS* (mg/100g)	RFS*(mg/100g)
Phenols (mg GAE/100g)	7.25±10.60	8.25±17.67*	1.8±0

(n=2); \*GFS- Germinated Flaxseeds; \*CFS- Control Flaxseeds; \*RFS- Roasted Flaxseeds; \* $P \ge 0.05$  (significance)

Table 3 Anti- Nutrients Composition of Flaxseeds

Anti- Nutrient	CFS* (mg/100g)	GFS* (mg/100g)	RFS*(mg/100g)
Tannins (mg GAE/100g)	3.4±4.24	2.95±2.12*	1.20±0.10
Phytic acid (mg)	46.8±0.00	34.0±0.00	2.4±0

(n=2); \*GFS- Germinated Flaxseeds; \*CFS- Control Flaxseeds; \*RFS- Roasted Flaxseeds; \* $P \ge 0.05$  (significance)

Table 4 Amylase Inhibition of the Flaxseeds

α- Amylase inhibition (U/L)	CFS*(U/L)	GFS*(U/L)	RFS*(U/L)
α- Amylase inhibition	80.1±0.76	85.47±0.75	86.01±1.52*

(n=2); \*GFS- Germinated Flaxseeds; \*CFS- Control Flaxseeds; \*RFS- Roasted Flaxseeds; \* $P \ge 0.05$  (significance)

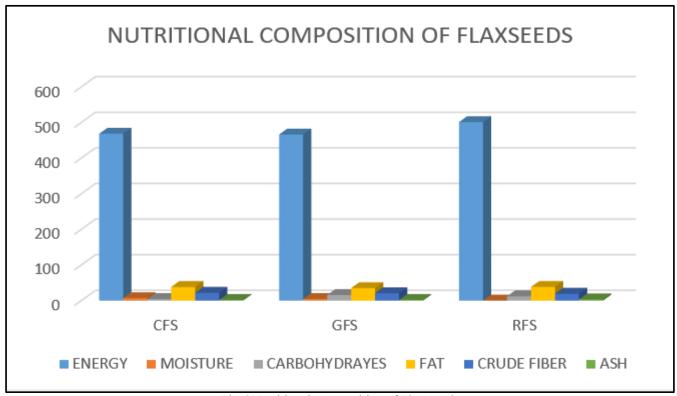


Fig 6 Nutritional Composition of Flax Seeds

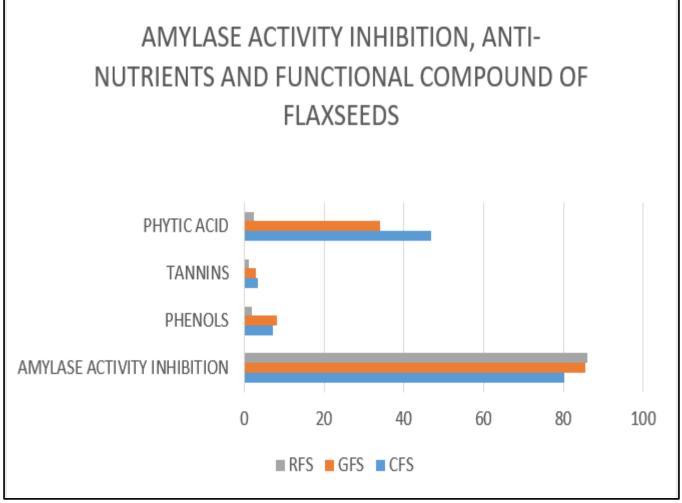


Fig 7 Amylase Activity Inhibition Anti- Nutrients and Functional Compound of Flaxseeds

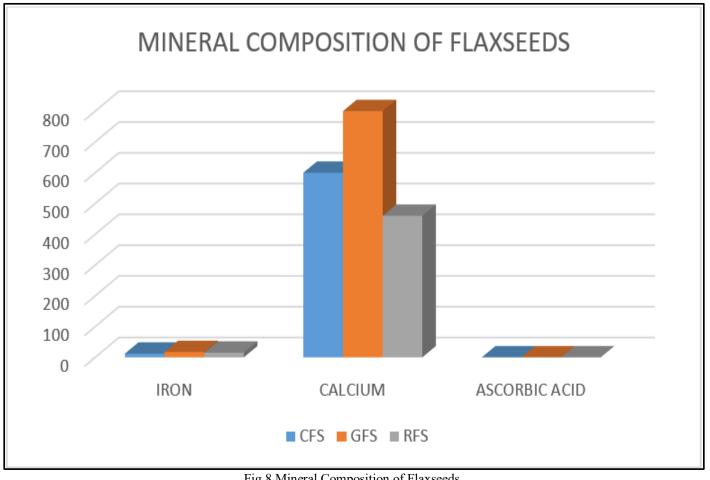


Fig 8 Mineral Composition of Flaxseeds

#### V. **CONCLUSION**

This study establishes that targeted processinggermination, roasting, and fermentation can decisively enhance the nutritional quality, functional potential, and bioactive profile of flaxseeds (Linum usitatissimum). Germination under cold conditions (5°C) activated enzymatic pathways that improved protein quality, minerals, vitamins and phenolics, while significantly reducing phytic acid and tannins. Low-flame roasting preserved antioxidant integrity and released bound phenolic acid and lignans, improving oxidative stability and nutrient concentration through moisture reduction. Fermentation with buttermilk- supported Lactobacillus activity which was confirmed by Grampositive bacterial identification. The combined application of these processes yielded synergistic gains in omega-3 fatty acid bioavailability, mineral uptake, protein digestibility, and sensory properties, while reducing anti-nutritional factors. These results highlight processing not as mere preservation but as a precision strategy to unlock and amplify flaxseed's intrinsic health benefits. Future studies should refine process parameters for maximal bioactive retention, assess stability of these compounds during storage, and validate their physiological impact through in vivo trials. Such efforts will ensure that processed flaxseeds reach their full potential as scientifically substantiated, high-value functional food ingredients.

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#### CONFLICT OF INTEREST

The author(s) declare no conflict of interest.

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