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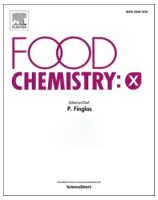

"Enhancement of flaxseed oil quality and yield using freeze-thaw pretreatment optimization: A novel approach"

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ABSTRACT

The impact of freezing-thawing (FT) pretreatment on flaxseed oil yield and quality was evaluated by pre-screening process parameters using a Taguchi design and further optimizing them through response surface methodology. The study examined freezing time (6–48 h), thawing time (6–24 h), and the number of cycles (1–5) on oil yield, thermal diffusivity, phenolic content, and antioxidants. The optimal conditions 6 h freezing, 6 h thawing, and 4 cycles resulted in a 50 % increase in oil yield and a 20 to 27 % improvement in antioxidants. Microstructural analysis showed surface disruptions at the cellular level, facilitating enhanced oil extraction. Additionally, the FT pretreatment increased thermal conductivity and diffusivity due to cracks and voids in the treated seeds. Furthermore, FT pretreatment did not affect the fatty acid profile or key physicochemical parameters (acid value 1.47 mg/KOH, peroxide value 2 meqO_2/kg, p-anisidine value 0.5 AnV), maintaining the oil's stability and quality. Therefore, FT pretreatment is an effective technique to enhance the oil yield and quality, providing a promising alternative to meet the growing global demand for edible oil.

1. Introduction

The global demand for edible oils has increased significantly, with domestic consumption reaching 43.5 million metric tons (USDA, 2024) and is projected to grow at a compound annual growth rate of 4.6 % by 2030 (Grand View Research, 2023). This growing demand needs efficient extraction techniques and enhanced oil quality to meet consumer and industrial requirements. Meeting this demand necessitates innovative approaches, such as identifying alternative oil sources or enhancing extraction technologies. Underutilized oilseed crops like linseed, rice bran, pistachio, and jatropha present promising alternatives (AOAC, 2005). Flaxseed or linseed (*Linum usitatissimum* L.), traditionally used in textile industry, remains underexplored option in the edible oil sector, largely owing to its high polyunsaturated fatty acid (PUFA) content (50–60 %), which limits its stability (Wang et al., 2022). However, it is rich in bioactive compounds such as lignans (secoisolariciresinol diglucoside), flavonoids, tocopherols, and phenolic acids, which exhibit antioxidant, anti-inflammatory, and cardioprotective properties (Alaubody et al., 2024).

Extraction technique is an important parameter that influences both the yield and quality of oil. Increasing the extraction temperature significantly enhances oil yield; however, it leads to the degradation of heat-sensitive bioactive compounds in the oil. Additionally, high temperature accelerates the oxidation of PUFAs, which can compromise the nutritional value and stability of the oil. Whereas, cold-press extraction operates at low temperatures, preserving sensitive bioactive compounds. However, this method results in lower oil recovery (24–26 % flaxseed oil yield), while retaining almost 15–30 % oil within seed cake (Melo et al., 2021). It is a relatively simple, rapid, cost-effective, and eco-friendly method. Whereas solvent extraction is an efficient method to obtain higher oil yield (40–45 %) but raises concern regarding both consumer health and the environmental impact of solvent disposal. In recent years, various pretreatments such as roasting, steam explosion, and enzymatic treatments have been induced before cold pressing to enhance oil yield and quality. However, these approaches often compromise oil's oxidative stability and nutritional value (Moknatjou et al., 2015). Emerging technologies, including ultrasound waves, micro/infrared radiation, and supercritical fluids significantly improve

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oil yield (Kulkarni et al., 2017). Due to technical complexity, these processing techniques pose significantly scalability challenges and can also alter oil's fatty acid profile (Szydłowska-Czerniak et al., 2020).

Freeze Thaw (FT) pretreatment approach have potential to address these issues. FT creates a thermal shock, disrupting cellular structure and enhancing antioxidant capacity and oil yield (Lee et al., 2020; Liu et al., 2021). FT induces cell disruption due to thermal shock due to ice crystals formed during the freezing process, which facilitates the release of bioactive compounds from the intercellular space rupture oleosomes, and release oil (Bramante et al., 2025). Additionally, this method operates at low temperatures and requires no chemicals or solvents. Recent studies have shown that the FT pretreatment can increase oil yield by 2.5 times in perilla seed oil (Lee et al., 2020). In an another studies where FT treatment were used in tiger nut seed oil, resulting in a 19.23 % higher oil yield (Zhang et al., 2024). Furthermore, studies such as by Jiao et al., 2018 reported enhancement of bioactive compounds like lutein and zeaxanthin increased by 14 folds and 14.4 folds, respectively in corn gluten meal after FT pretreatment. Despite the growing interest in FT pretreatment, its impact on the oxidative properties, physicochemical properties, and fatty acid composition of flaxseed oil is still underexplored. Additionally, the structural and thermal characteristics of flaxseed during the FT also remain poorly understood, highlighting the need for further research to optimize this method. This study aims to address these gaps with this specific objective of optimizing freeze thawing parameters with respect to oil yield and quality.

2. Materials and methods

2.1. Materials

Flaxseeds *(Linum sativum)* of NL-260 variety were collected from Barsar, Himachal Pradesh, India. The impurities like stones, husk, and dust were removed manually, and the seeds were dried for 48 h at 35 °C using a laboratory hot air oven (Macro Scientific Works Pvt. Ltd., India). The dried seeds were packed in zip lock bags and stored at 4 °C. All the chemicals and gases used were of analytical grade, and borosil glassware was used.

2.2. Physical dimensions and engineering properties of flaxseeds

The geometric measurements of seed shape, size, and thickness, geometric mean diameter, sphericity, and gravimetric properties such as true density, bulk density, and static coefficient of friction were determined following the method outlined by Satpathy et al. (2024).

2.3. Experimental procedure

In this study, FT pretreatment was carried out by multiple factors that might influence the process. A Taguchi orthogonal array was used to screen the key factors influencing the FT pretreatment. The selected factors were further optimized by the process parameters using response surface methodology Box-Behnken design (RSM-BBD) to ensure precision in maximizing oil recovery while maintaining the highest possible quality standards. The seeds obtained after the FTO (Freeze-thaw optimized) process were characterized for their structural properties, engineering, and thermal properties, while the extracted oil obtained was analyzed for oil yield, physicochemical properties, antioxidant properties, and fatty acid profile to assess changes as compared to the untreated flaxseed (UF) and untreated flaxseed oil (UFO).

2.3.1. Freeze-thaw pretreatment

In the present study, 200 g of seeds (5 % moisture) were packed in a low-density polyethylene (LDPE) bag, and 200 mL of distilled water was added to the bag, followed by freezing at −20 °C. The frozen seeds were thawed at 4 °C and then refrozen again (Lee et al., 2020). The process was repeated in multiple cycles. In the above process, the freezing time (Ft), freezing temperature (Ftem), thawing time (Tt), thawing temperature (Ttem), and number of cycles (N) varied according to the runs provided design of the experiment in Table 1 and Table 3. In the end, the seeds were oven-dried at 35 °C and conditioned to a 5 % moisture level before pressing.

2.3.2. Pre-screening using Taguchi L-8 orthogonal array integrated with multi-response signal-to-noise (MRSN) ratio approach

Taguchi method was used to identify the key parameters affecting the FT process, as there were many factors that could affect the process (Shafizah et al., 2022). This involved a systematic investigation of five factors: freezing time (Ft), freezing temperature (Ftem), thawing time (Tt), thawing temperature (Ttem), and the number of cycles (N). These parameters and their respective levels were systematically varied across eight runs, as structured by the Taguchi design, to capture preliminary response metrics such as oil yield, peroxide value, and free fatty acid. The results from this pre-screening phase are summarized in Table 1. Identification of the key parameters was achieved by integrating multi-response signal-to-noise (MRSN) ratios across all responses generated from the Taguchi L-8 orthogonal array. Initially, the quality loss (L_{ij}) for each response is calculated. For larger-the-better responses, such as extracted oil yield (%), L_{ij} is calculated using equation below:

$$L_{ij} = \frac{1}{n_i}\sum_{k=1}^{n_i} 1 \Big/ y_{ijk}^2 \tag{1}$$

Similarly, for smaller-the-better responses such as peroxide value (PV) and free fatty acids, L_{ij} is calculated using equation below:

$$L_{ij} = \frac{1}{n_i}\sum_{k=1}^{n_i} y_{ijk}^2 \tag{2}$$

Where, L_{ij} represents the quality loss function for the i^{th} response at the j^{th} trial. The term y_{ijk} denotes the observed data for the i^{th} response at the j^{th} trial and the k^{th} repetition and n_i refers to the number of replications for the i^{th} response. Since each response is measured on a different scale, L_{ij} is further normalized to a scale ranging from 0 to 1 using the normalized quality loss function (C_{ij}), as described in the equation below:

$$C_{ij} = \frac{L_{ij}}{max\left(L_{i1}, L_{i2}, \ldots, L_{ij}\right)} \tag{3}$$

Subsequently, the total normalized quality loss (TNQL) was computed by summing the C_{ij} values for all three response variables associated with each experimental trial. Finally, the MRSN ratio for each trial is calculated using equation follows:

$$MRSN\_j = -10\, log\left(TNQL\_j\right) \tag{4}$$

The optimal combination of factors and levels was identified by minimizing the expected quality loss. The factors were then ranked based on the absolute magnitude of ΔMRSN, with higher values indicating a greater influence on the overall response (Table S1). Based on the results obtained from this design, an optimization experiments using the Box-Behnken Design (BBD) was used to refine the conditions identified and as most influential during the pre-screening. The optimization trials were specifically designed to determine the optimal combination of process parameters.

2.3.3. Experimental design for RSM-BBD

The further optimization of the pre-screened process variables was conducted by setting up experiments based on a 3-factor, 3-level Box-Behnken design matrix Table 2 and analyzed using response surface methodology (RSM). Based on the pre-screening experiments (Table 1), the three most influential factors freezing time (Ft), thawing time (Tt), and number of cycles (N) with their corresponding levels, and constant freezing temperature of −20 °C and 4 °C thawing temperature were selected for optimization using RSM (Mane et al., 2024).

Table 1
L8 Taguchi orthogonal array for pre-screening variables in the freeze-thaw process.

Run	X1 (hrs)	X2 (°C)	X3 (hrs)	X4 (°C)	X5 (cycles)	Oil yield (%)	Peroxide value (mEq/O_2)	FFA (%)	TNQL	MRSN ratio
1	48	-20	6	4	5	34.30 ± 2.15	1.51 ± 0.06	0.28 ± 0.01	0.31	5.02
2	6	−80	6	4	1	24.47 ± 1.42	1.50 ± 0.03	0.50 ± 0.02	0.49	3.10
3	6	−20	24	4	1	31.92 ± 1.37	1.50 ± 0.03	0.40 ± 0.01	0.34	4.63
4	48	−80	24	27	1	32.78 ± 1.26	1.53 ± 0.04	0.60 ± 0.02	0.36	4.48
5	6	−20	24	27	5	31.40 ± 2.14	2.10 ± 0.02	1.71 ± 0.05	0.72	1.45
6	6	−80	6	27	5	31.41 ± 0.81	2.35 ± 0.06	1.98 ± 0.08	0.87	0.62
7	48	−80	24	4	5	30.95 ± 1.52	1.49 ± 0.06	1.73 ± 0.04	0.59	2.26
8	48	−20	6	27	1	29.92 ± 2.23	1.53 ± 0.06	0.29 ± 0.01	0.37	4.29

X1 represents freezing time; X2 represents freezing temperature; X3 represents thawing time; X4 represents thawing temperature, X5 represents the number of cycles, Free fatty acid (FFA) as oleic acid, TNQL stands for total normalized quality loss; MRSN ratio stands for multi-response signal-to-noise ratio.

Table 2
Parameters for optimization of freeze-thaw process.

Independent variables		Coded levels		
Parameters	**Code**	−1	0	+1
		Actual levels		
Freezing Time (FT) (hrs)	A	6	27	48
Thawing Time (TT) (hrs)	B	6	15	24
Number of cycles (N)	C	1	3	5
Dependent variables		**Constant Variables**		
Oil yield (%)	R1	Freezing temperature	−20 °C	
Thermal diffusivity (mm^2/s)	R2			
TPC (mg GAE/100 g)	R3	Thawing temperature	4 °C	
DPPH radical scavenging (%)	R4			

A total of 17 experimental runs, with three independent variables, namely Ft, Tt, and number of cycles, having three levels of each varying from 6 to 48 h, 6 to 24 h, and 1 to 5 cycles respectively, were conducted. All runs were randomized to reduce the possibility of unpredicted deviations. From these experiments, four different response variables, oil yield (%), thermal diffusivity (mm^2/s), total phenolic content (TPC) (mg GAE/100 g), and 2,2 Diphenyl-1-picrylhydrazyl DPPH radical scavenging activity (%) were measured in triplicate, and the mean was recorded as the single representative value in the design matrix Table 2.

2.3.4. Analysis of response parameters

Oil yield.

Before pressing, the FT-treated flaxseeds were placed in desiccators at room temperature to ensure uniform moisture content. This step helps to reduce variation in seed moisture content that might impact the oil yield. Afterward, 200 g of seeds were transferred into the oil expeller (SONAR, Delhi, India). The resulting flaxseed oil (FO) was filtered using Whatman No. 42. The oil was collected into dark glass containers and stored at 4 °C for further analysis. The yield of the oil was calculated using a gravimetric method as follows:

$$\text{Oil yield}\ (\%) = \frac{W1}{W2} \times 100 \quad (5)$$

W2 is the total seed weight, and W1 is the weight of oil extracted from seeds.

Thermal diffusivity of flaxseed.

The thermal diffusivity and thermal conductivity of flaxseed samples were measured using the Hot Disk® thermal constants analyzer (TPS 500, Göteborg, Sweden) equipped with a Kapton 5501 F2 disk sensor. Flaxseed samples were placed in a sample container, ensuring good contact with the sensor, which was positioned between two layers of the sample. Measurements were conducted with a 10-s transient recording. The analyzer simultaneously served as a heat source and a dynamic temperature sensor, recording the temperature increase over time. The instrument's software calculated thermal diffusivity from the recorded data (Nabil & Khodadadi, 2013).

Antioxidant potential (Total phenolic content and DPPH radical scavenging activity percentage %).

A liquid-liquid extraction method was employed to extract polar compounds from the oil. For this, 0.5 g of oil was dissolved in 3 mL of a methanol-water solution (80:20, *v*/v). The mixture was thoroughly shaken and then centrifuged at 6000 rpm for 5 min to separate the polar extract. This procedure was repeated three times, with the supernatants from each cycle combined to achieve a final volume of 10 mL, using the same 80:20 methanol-water solution for further analysis. Now in the 0.5 mL of methanolic extract, 2.5 mL Folin–Ciocalteu reagent (10 %) was added and incubated for 3 min. Then, samples were treated with 2 mL of 7.5 % Na_2CO_3 for 15 min at room temperature and incubated for 3 h. The measurements were performed at 765 nm using a UV-VIS-1800 SL-159 spectrophotometer (Kyoto, Japan) and expressed in gallic acid equivalent (mg GAE/100 g) (Singh et al., 2024).

The DPPH radical scavenging activity of the oil samples was assessed using a modified version of the method described by (Singh et al., 2024). In this procedure, 2 mL of the oil's polar extract was mixed with 2 mL of a 0.2 mM methanolic DPPH solution. The mixture was incubated in the dark for 30 min, after which the absorbance at 517 nm was measured using a Shimadzu UV-VIS-1800 SL-159 spectrophotometer with the absorbance of the blank reagent subtracted. The antioxidant activity was calculated as the percentage of inhibition using the following formula:

$$\%\text{DPPH radical scavenging activity} = 100 \times (A_o - A)/A_o \quad (6)$$

where, A represents the absorbance of the sample solution and the A_0 absorbance of the control sample.

2.4. Characterization of seeds

2.4.1. Morphological analysis

The microstructure of flaxseeds was analyzed using a scanning electron microscope (SEM) (JSM-6700F, Jeol, Japan) following a modified preparation method described by Lee et al. (2020). The flaxseed samples were mounted on SEM stubs using double-sided carbon tape for stability. The samples were then sputter-coated with a thin layer of gold to enhance conductivity. Imaging was conducted using an accelerating voltage of 15 kV in back-scatter mode.

2.4.2. Thermal properties

Thermal properties of seeds obtained through optimized process were analyzed as per the procedure mentioned in section 2.3.4.

2.4.3. Oil yield

The oil yield of the optimized process was calculated similar to procedure mentioned above in 2.3.4.

2.5. Characterization of oil

2.5.1. Physicochemical properties

The UFO and optimized flaxseed oil (FTO) were analyzed for physicochemical properties such as peroxide value (PV), acid value (AV), free fatty acid (FFA), p-anisidine (p-Av), color, refractive index, and viscosity (Singh et al., 2024). The refractive index of oils was determined at 40 °C using an Abbe refractometer (Atago RX-7000i, Japan). The color of the oil samples was analyzed for R(redness), Y(yellowness), and N(neutral) using R Lovibond tintometer (The Tintometer ltd., UK). The free fatty acid, peroxide value, and p-anisidine value content were determined using the AOAC Ca 5a-40, Cd 8b-90, and Cd 18–90, respectively (AOAC,2005).

2.5.2. Fatty acid composition analysis

Fatty acid methyl esters were synthesized using a derivatization procedure as described by (AOCS, 1997). The analysis was conducted using the Compass CDS software with a column set to the following temperature program: initial temperature of 120 °C with a hold time of 1 min, ramping at 10 °C/min to 175 °C with a hold time of 10 min, then at 5 °C/min to 210 °C with a hold time of 10 min, and finally at 5 °C/min to 230 °C with a hold time of 9.5 min. The equilibration time was 0.5 min, and the maximum temperature was 260 °C. The syringe volume was 10 μL, with an injection volume of 1 μL and an injection dispense speed of 6000 μL/min. The column specifications were slightly modified. FAME analysis was performed using a flame ionization detector (FID).

2.5.3. Antioxidant activity

The DPPH radical scavenging activity percentage (%) and TPC were determined as per the procedure outlined in section 2.6.1. The ABTS inhibition percentage (%) was conducted by preparing an ABTS stock solution in methanol. The ABTS radical cation was generated by oxidizing a 7 mM ABTS solution with 2.45 mM potassium persulfate. The mixture was kept in dark place for 16 h incubation time at room temperature. 1 mL of ABTS diluted solution was mixed with 10 μl of the sample at the different concentrations for 6 min, and then absorbance was measured at 734 nm. The percentage drop in the absorbance values was reported as DPPH radical scavenging activity or ABTS free radicals (Singh et al., 2024).

$$\text{ABTS inhibition\%} = 100 \times (A_o - A)/A_o \quad (7)$$

where, A represents the absorbance of the sample solution and the A_0 absorbance of the control sample.

The FRAP (Ferric reducing antioxidant power) assay was used to assess the antioxidant activity of the test samples. The FRAP reagent was freshly prepared by combining 10 mM 2,4,6-Tripyridyl-*s*-triazine (TPTZ) dissolved in 40 mM HCl, 250 mM sodium acetate buffer (pH 3.6), and 20 mM $FeCl_3 \cdot 6H_2O$ in a 1:1:10 ratio. To assay was performed as per the method by Ghosh et al. (2019) with slight modification. 1 μL of the sample extract was mixed with 9 μL of the FRAP reagent to perform the assay. The mixture was then incubated at 37 °C for 4 min, and the absorbance was recorded at 593 nm.

2.6. Statistical analysis

All experiments were carried out in triplicate, and the findings were reported as the mean ± standard deviation. Pre-screening data were analyzed using Excel 2021 (Microsoft 365). Multivariate optimization and data analysis, specifically analysis of variance (ANOVA) for independent and dependent parameters, were conducted by applying Response Surface Methodology (RSM) with Design Expert (version 13, Stat-Ease Inc.).

2.6.1. Optimization and model validation

The second-order polynomial equation was solved using a statistical approach to fit a mathematical model to a set of experimental data. The results of regression analysis were obtained in terms of ANOVA, regression coefficient, and associated statistics. Optimization of all the independent parameters with respect to the chosen response was done using RSM. The goal was set for significant dependent and independent variables as per the required criteria of variables. Among all the optimized solutions (as given by software) the best optimum solution for optimum value of independent variables were selected on the basis of criteria that the optimal value should be closed to variable value and higher desirability.

Graphical analysis was done using 3D diagrams showing the effect of independent variables on responses. The combination of the independent variables was selected by keeping either variable at the center was selected by keeping either variable at the center value obtained during numerical optimization. Experiments were conducted to validate the optimum results as given by the software. The optimize value were verified with actual values and compare to determine validity of model and optimal results.

3. Results and discussion

3.1. Pre-screening using Taguchi L-8 orthogonal array integrated with multi-response signal-to-noise (MRSN) ratio approach

The Taguchi L-8 orthogonal design matrix was utilized to systematically explore the crucial factors affecting the FT process of flaxseeds. Five key parameters were studied: freezing time, thawing time, freezing temperature, thawing temperature and the number of cycles. Each set of parameters was tested under consistent conditions. The initial findings, outlined in Table 1, revealed notable differences in oil yield (%), peroxide value (meq/Kg), and free fatty acid value. ΔMRSN is a single numerical metric that quantifies the overall quality of a system or process when multiple responses are considered simultaneously with different target outcomes (Gauri & Pal, 2017). The results indicate that a number of cycles had the greatest influence on the system, as evidenced by the highest ΔMRSN value of −1.79, leading to its Rank 1 position in Table S1. The ΔMRSN for cycles suggests that a lower treatment level yielded better performance. This could be due to reduced chemical changes occurring at lower cycles as compared to a higher number of cycles (Boerner et al., 2020). Freezing time and thawing time also showed significant effects, with ΔMRSN values of 1.56 and 1.24, respectively. The positive ΔMRSN values for these factors suggest that higher levels of freezing time and thawing time contributed positively to the overall performance but to a lesser extent than cycles. These results underscore the importance of optimizing processing time and number of cycles to achieve the desired product quality.

3.2. Influence of independent variables on the dependent variables

From Taguchi design, the three most important factors affecting the quality and quantity of FO were selected for the RSM-BBD. BBD was chosen to examine the linear and interactive effects of independent variables freezing time, thawing time, and number of cycles on the dependent variables: oil yield, thermal diffusivity, TPC, and DPPH radical scavenging activity (%) mentioned in Table 2.

3.2.1. Oil yield

Oil yield is the crucial factor influencing the production and economic viability of the oil extraction process in industries (Lavenburg et al., 2021). Higher oil yield maximizes resource utilization, enhances production efficiency, and improves food security and sustainability goals by reducing environmental stress. The oil yield after FT treatment ranges from 25.12 to 39.11 %. The highest oil yield of 39.11 % was found at 27 h freezing time, 24 h thawing time, and 5 cycles, whereas the

lowest oil yield was 25.12 % at 27 h freezing time, 6 h thawing time, and 1 cycle (Table 3). There was an increase in oil yield from 26.4 to 38.5 % oil when the number of cycles increased from 1 to 5. In this, both the linear and quadratic terms were positive, indicating a sharp increase in oil yield with the number of cycles. This increase is attributed to the enhanced cell wall disruption changes characterized by an increase in intercellular spaces due to cell shrinkage, enhanced porosity, reduced density, and cell wall disruption caused by ice crystal formed during freezing with each additional freeze-thaw cycle, leading to greater structural breakdown and thus higher oil extraction (Tu et al., 2021). Similar results with FT pretreatment were obtained in the case of perilla seed extraction when treated with FT pretreatment (Lee et al., 2020). The linear term showed a positive effect for thawing time and a negative for the quadratic term. This negative relation at quadratic term shows that longer thawing time has negative effect in the oil yield. This suggests that initial thawing enhances oil release by facilitating cell wall disruption, but excessive thawing can lead to oil loss, and increased oxidation or microbial activity, ultimately reducing oil quality and yield. Similar trends with excessive thawing were also reported by Rahman et al. (2015). Additionally, excessive cellular breakdown may create a more liquid consistency, making oil separation from cell debris more difficult. A similar effect was observed in the extraction of phycobiliproteins by Tan et al. (2020). Specifically, thawing for 24 h resulted in the highest extraction yield of phycobiliproteins, with a total yield of 219.87 ± 0.68 mg/g from *Arthrospira* sp. as longer thawing gives an adequate amount of time to break cells and easier extraction of compounds whereas, shorter thawing times (e.g., 2 h) yielded significantly lower amounts of phycobiliproteins, often less than 15 mg/g. All the significant interactions were best represented by a quadratic polynomial equation:

$$\text{Oil yield (\%)} : 34.00 + 0.5505 \times {}_1 + 1.05 \times {}_2 + 5.50 \times {}_3 + 1.15 \times {}_1{}^2 + 0.65 \times {}_2{}^2 – 2.75 \times {}_3{}^2 \quad (8)$$

X_1, X_2, X_3 are freezing time, thawing time and number of cycles respectively.

3.2.2. Thermal diffusivity

The thermal diffusivity method is a highly effective and widely used viability test for determination of injury in different plant parts after freezing treatment. The duration of freezing showed a positive linear effect and a negative quadratic effect. Thermal diffusivity was found to be a minimum of 1.95 mm^2/s at 6 h Ft, 15 h Tt, and 1 cycle, while it was a maximum of 5.06 mm^2/s at 48 h Ft, 15 h Tt, and 5 cycles. Thermal diffusivity increased from 3.5 to 4.1 mm^2/s as freezing time increased, resulting in a higher freezing rate and more compact ice crystals forming, which facilitated heat transfer. The number of cycles positively affected both linear and quadratic levels. Thermal diffusivity significantly increases from 2.2 to 4.7 mm^2/s as the number of cycles increases. This was due to cell disruption creating smaller segments, allowing for easier diffusion paths. Specific conductivities increased with the number of segments in a sample, as larger segments exhibited lower thermal diffusivity due to heat diffusion within the cell (Prášil, & Zámečník, J., 1998). Similarly, thawing time had a positive effect at the linear level and a negative at the quadratic level, with a slight decrease in thermal diffusivity as thawing time increased. There is an increase in thermal diffusivity with thawing time initially, but prolonged thawing negatively impacts it due to weakened structural stability of the cells, leading to higher thermal resistance. Additionally, as seeds become saturated with released water and oil, heat transfer efficiency declines, hindering uniform heat conduction and reducing the material's ability to spread heat effectively.

All the significant interactions were best represented by a quadratic polynomial equation:

Thermal diffusivity (mm^2/s): $3.78 + 0.2804 \times {}_1 + 0.0579 \times {}_2 + 1.26 \times {}_3 + 0.0191 \times {}_1^2 – 0.1259 \times {}_2^2$.

$$^{-}0.2951 \times {}_3{}^2 – 0.0257X_1X_2 – 0.005X_1X_3 – 0.04X_2X_3 \quad (9)$$

X_1, X_2, and X_3 are freezing time, thawing time, and number of cycles, respectively.

3.2.3. Antioxidant potential and total phenolic content

Oils with better antioxidant capacity are particularly important due to their health benefits, producing better nutritional quality and stability. The TPC ranges between 55.23 and 80.42 mg GAE/100 g, and DPPH radical scavenging activity from 41.17 to 50.40 %. The lowest TPC was found at 6 h freezing, 15 h thawing and 1 cycle, whereas the maximum phenolics were found at 48 h freezing time, 24 h thawing time and 3 number of cycles. Similarly, the minimum DPPH radical scavenging activity was found at 27 h freezing time, 6 h thawing time and 1 cycle, while the maximum DPPH radical scavenging activity was noted at 48 h freezing, 24 h thawing and 3 cycles. In FTO process there was a positive linear relationship observed between thawing time and the number of cycles concerning the antioxidant properties of FO. Increasing thawing time led to an increase in TPC from 76 to 79 mg GAE/100 g and an increase in DPPH radical scaven39,1ging activity from 48 % to 50 %.

Table 3
Box-Behnken experimental design for freeze-thaw process.

Run	Factor 1	Factor 2	Factor 3	Response 2	Response 1	Response 3	Response 4
	Freezing Time (°C)	Thawing Time (°C)	Number of cycles	Oil yield (%)	Thermal Diffusivity mm^2/s	TPC mg GAE/100 g	DPPH radical scavenging %
1	48	24	3	38.04	3.9	**80.42***	**50.40**
2	48	6	3	36.11	4.02	75.4	49.02
3	27	15	3	34.04	3.75	76.14	49.11
4	27	15	3	35.2	3.8	76.21	49.10
5	6	15	5	37.9	4.49	75.11	47.2
6	27	15	3	35.2	3.8	78.02	50.21
7	27	24	1	28.02	2.03	62.05	44.11
8	27	24	5	**39.11**	4.6	74.01	47.32
9	6	15	1	27.23	**1.95**	**55.23**	41.75
10	48	15	1	29.11	2.5	60.33	43.53
11	48	15	5	38.22	**5.06**	74.11	47.21
12	6	24	3	37.04	3.287	79.27	50.01
13	27	15	3	35.1	3.75	78.31	48.67
14	27	6	1	**25.12**	2.19	56.55	**41.17**
15	6	6	3	34.6	3.49	77.43	48.54
16	27	6	5	37.3	4.6	77.12	48.31
17	27	15	3	34.23	3.78	76.54	49.23

* lowest and the highest values.

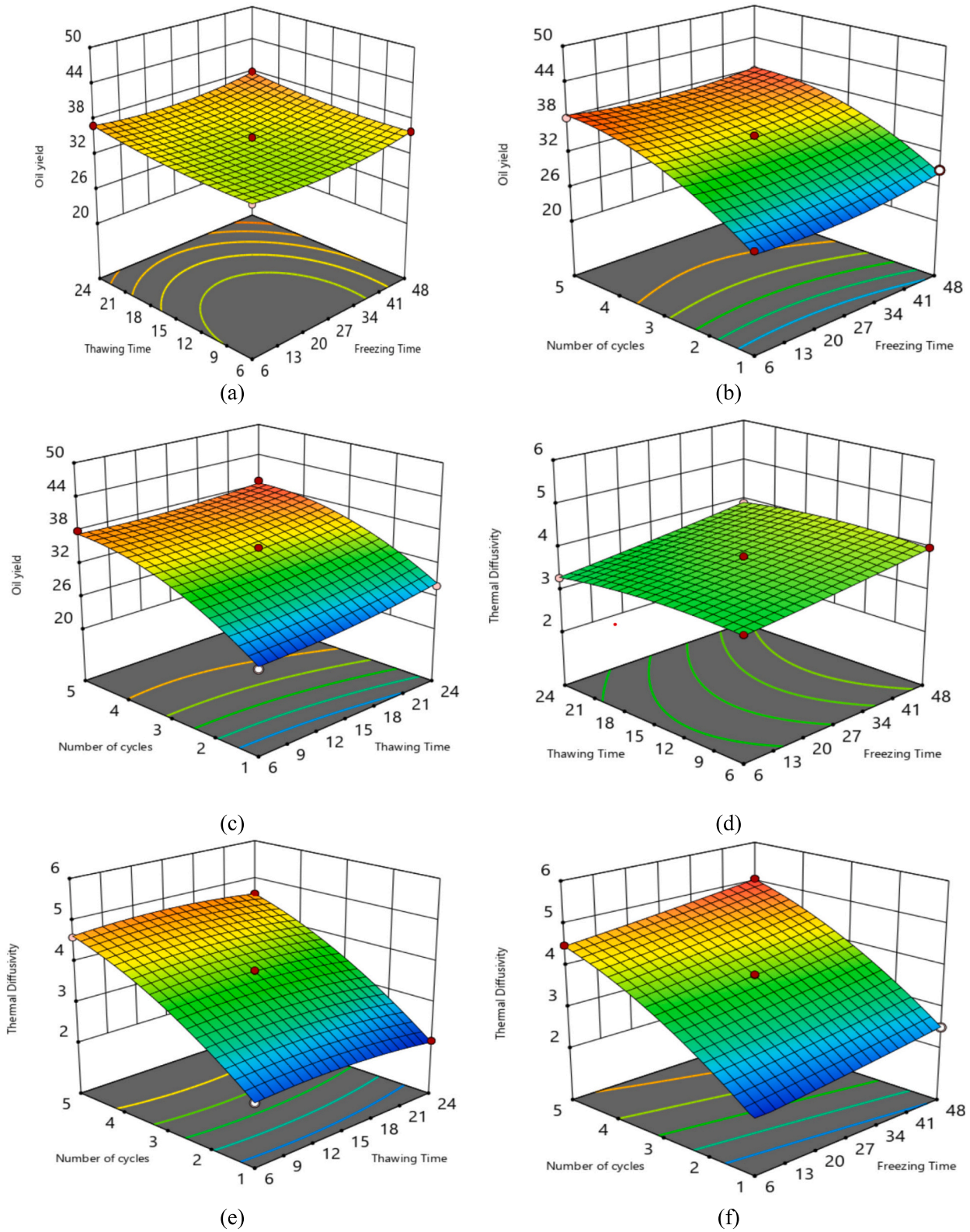


Fig. 1. 3 D interactive graphs **(A)** Effect of Freezing time and Thawing time on oil yield **(B)** Effect of Thawing time and number of cycles on oil yield **(C)** Effect of Freezing time and number of cycles on oil yield, graphs **(D)** Effect of Freezing time and Thawing time on thermal diffusivity **(E)** Effect of Thawing time and number of cycles on thermal diffusivity **(F)** Effect of Freezing time and number of cycles on thermal diffusivity **(G)** Effect of Freezing time and Thawing time on oil total phenolic content (H) Effect of Thawing time and number of cycles on total phenolic content **(I)** Effect of Freezing time and number of cycles on total phenolic content **(J)** Effect of Freezing time and Thawing time on DPPH radical scavenging activity % **(K)** Effect of Thawing time and number of cycles on DPPH radical scavenging activity % **(L)** Effect of Freezing time and number of cycles on DPPH radical scavenging activity %. Design expert 13.0 was used to illustrate the diagram.

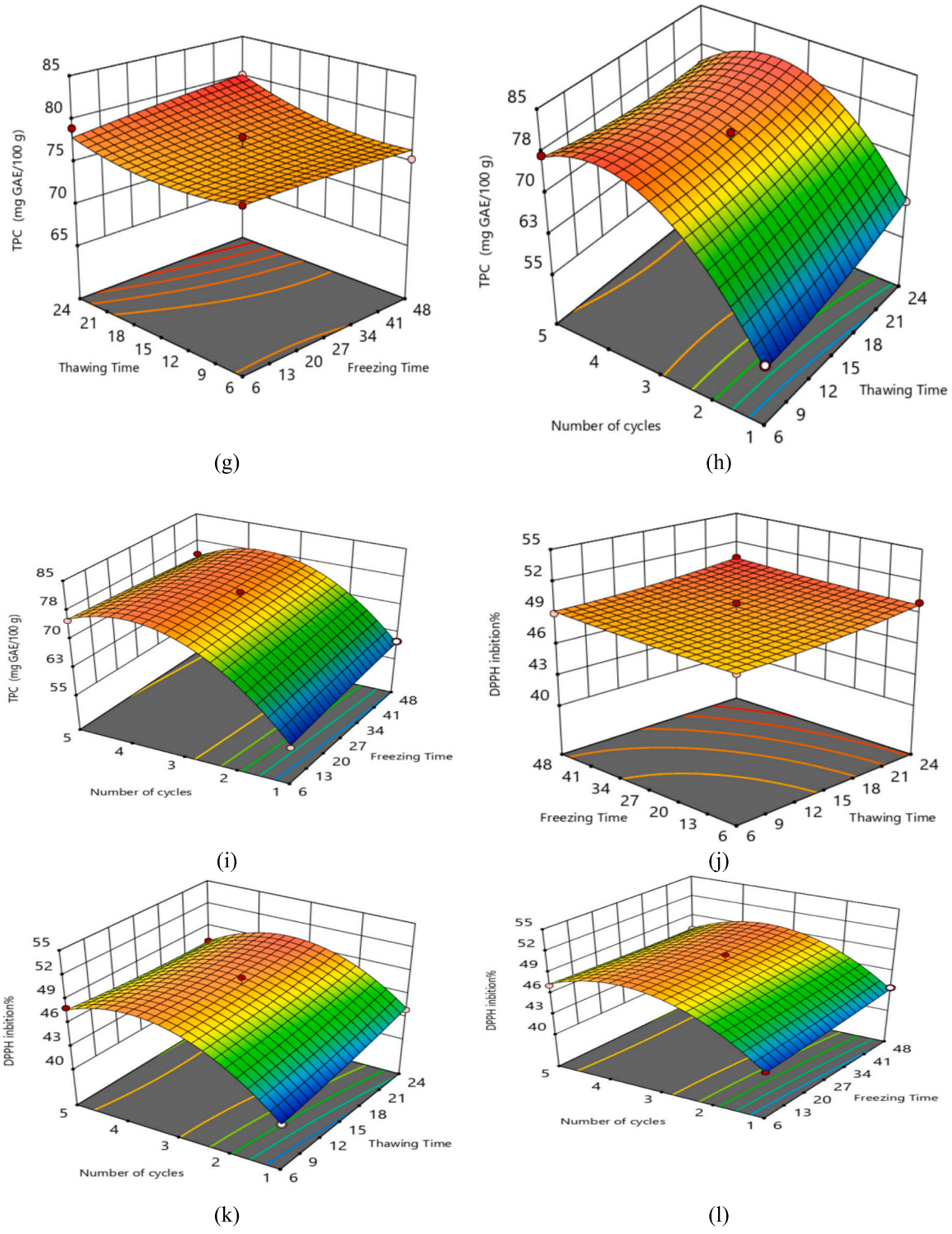


Fig. 1. (*continued*).

This effect can be attributed to the leaching of extracellular bioactive materials during thawing, facilitating the release of antioxidants from the plant-based raw material (Zhao et al., 2015). However, prolonged thawing degrades antioxidant quality as it increases oxygen exposure and enzymatic activity (e.g., polyphenol oxidase), leading to the oxidation of phenolics and vitamins. Heat and water exposure during longer thawing further destabilize antioxidants through hydrolysis and degrade antioxidant compounds (Phinney et al., 2017). Similarly, an increase in the number of cycles resulted in higher TPC values, rising from 57.8 to 80.15 mg GAE/100 g, and enhanced DPPH radical scavenging activity values from 42.39 to 50.04 %. The repeated melting and reformation of ice crystals during cycles could lead to structural collapse or mechanical damage, releasing more antioxidants from the disrupted seeds. Comparable findings were observed in the extraction of carotenoid contents where decline in TPC was noted after four cycles, which may indicate excessive degradation of phenolic compounds due to increased injury (Benjakul & Bauer, 2001). There was significant interactive effect at $p < 0.05$ of BC and AC on TPC, whereas the

interaction was not significant for AB at $p < 0.05$. The 3D graph shows that the TPC was higher at around 48 h of freezing time and 4 cycles and decreased after 4 cycles, declining thereafter, possibly due to the destruction of phenolic compounds due to enzymatic action or oxidative reaction (Zhang et al., 2021). The interactive graph of BC shows that the maximum phenolic were obtained at 4 cycles and approximately 48 h thawing time. However, the interactive effect graph for AB did not show significance ($p < 0.05$) in terms of TPC. Fig. 1 shows the response surface plots depicting the relationship between thawing time and number of cycles and their mutual effect on the DPPH radical scavenging activity. Statistical analysis confirmed that the interaction of thawing time and number of cycles significantly influenced DPPH radical scavenging activity ($p < 0.05$), with the highest DPPH radical scavenging activity achieved at 4 cycles and 24-h thawing time. That might be because both thawing time and a number of cycles increase the antioxidant capacity till certain points, but prolonged thawing resulted in the degradation of antioxidant compounds such as phenols, which might be due to oxidative or enzymatic stress. In contrast, interactions AC and AB were not significant at $p < 0.05$. The interactive model did not show significance for oil yield at $p < 0.05$. All the significant interactions for TPC and DPPH radical scavenging activity were best represented by a quadratic polynomial equation:

Total phenolic compounds (mg GAE/100 g)

$$: 76.80 + 1.16 \times {}_2 + 8.30 \times {}_3 - 10.64 \times {}_3{}^2 - 1.50X_1X_3 \quad (10)$$

DPPH radical scavenging activity (%)

$$: 49.13 + 0.3063 \times {}_2 + 2.37 \times {}_3 - 1.00X_2X_3 - 4.38 \times {}_3{}^2 \quad (11)$$

X_1, X_2, X_3 are freezing time, thawing time and number of cycles respectively.

3.3. Response surface modelling (BBD)

A strong correspondence between the R^2, R^2-adjusted, and R^2-predicted values (Table 4), all of which are close to 1 for each response: 0.991, 0.981, and 0.930 for oil yield; 0.999, 0.998, and 0.994 for thermal diffusivity; 0.9939, 0.9861, and 0.9670 for TPC; and 0.983, 0.961, and 0.843 for DPPH radical scavenging activity. These results indicate a robust linear relationship between the predicted and experimental values, which confirms a strong correlation between the obtained model and the responses. The data for each response was fitted to a second-order polynomial equation, and ANOVA analysis was performed to assess the significance of the model. The significance of the model terms was indicated by the F-value or *p*-value, with ($p < 0.05$) and non-significance ($p > 0.05$) of the model terms in Table 4. The models were significant at F-value of 94.45 ($p < 0.01$) for oil yield, 1562.61 ($p < 0.01$) for thermal diffusivity, 126.81 ($p < 0.0001$) for TPC, and 45.76 for DPPH radical scavenging activity, indicating the models are statistically significant. Additionally, the quadratic model was significant ($p < 0.0001$) with an insignificant lack of fit with lower residual values (<2.5) and extremely low coefficients of variation (CV), which indicates low variance in the means, confirming the accuracy of the results.

3.3.1. Optimization and model validation

The quadratic models established for each response variable oil yield, thermal diffusivity, TPC, and DPPH radical scavenging activity was utilized to forecast the optimal values of the independent variables. The goal was to maximise the dependent parameters while minimizing the independent parameters (freezing time and thawing time) to conserve time and energy. The number of cycles were kept within an acceptable range (Table 4). Following the optimization of each response individually, a composite solution was obtained by computing the geometric mean of the desirability for all responses. The most desirable solution identified the optimal values for both the independent and dependent variables. The optimized conditions for maximum oil yield of 38.28 %, thermal diffusivity of 4.167 mm^2/s, TPC of 80.15 mg GAE/100 g and DPPH radical scavenging activity of 49.14 % were determined to be a freezing time of 6 h, a thawing time of 6 h, and 4 cycles, with model desirability of 90.8 %. Each model was validated with the optimal experimental points. Results were collected in triplicate, and the means were statistically compared to the predicted values from the models as well as to the control group. The means were analyzed for statistical significance ($p < 0.05$) against the predicted and control values. Table 5 shows the predicted and experimental values of the process variables, demonstrating a non-significant difference with only a small variation in the means.

Table 4
ANOVA for three-factor response model in the freeze-thaw process.

Source	df	Oil yield (%)		Thermal diffusivity mm^2/s		TPC (mg GAE/100 g)		DPPH radical scavenging activity %	
		Mean Square	F-value	Mean Square	F-value	Mean Square	F-value	Mean Square	F-value
Model	9	31.47	94.45	1.54	1562.61	118.66	126.81	14.58	45.76 ^{s}
A	1	2.42	7.26^s	0.62	640.11^s	12.50	13.36 ^{s}	4.56	14.31 ^{s}
B	1	11.28	33.86 ^{s}	0.026	27.27^s	3.13	3.34	2.51	7.87 ^{s}
C	1	230.05	690.4^s	12.70	12,927.5^s	561.13	599.68 ^{s}	44.75	140.42
AB	1	0.04	0.12	0.002	2.70	0.00	0.00	0.11	0.34
AC	1	1.00	3.00	0.0001	0.101	9.00	9.62 ^{s}	0.98	3.08 ^{s}
BC	1	1.00	3.00	0.006	6.51 ^{s}	12.25	13.09 ^{s}	4.0	12.55
A^2	1	5.57	16.71^s	0.001	1.57	0.318	0.340	0.025	0.078
B^2	1	1.78	5.34	0.066	67.90 ^{s}	4.00	4.28	0.012	0.037
C^2	1	31.84	95.56^s	0.366	373.28 ^{s}	466.42	498.47 ^{s}	73.81	231.65 ^{s}
Residual	7	2.33		0.006		0.935		0.318	
Lack of Fit	3	1.13	1.26	0.004	2.00	0.583	0.48	0.403	1.5
Pure Error	4	1.20		0.0025		1.20		0.254	
Std. Dev.		0.577		0.0313		0.9673		0.564	
Mean		34.15		3.59		72.18		47.15	
CV %		1.69		0.873		1.34		1.20	
R^2		0.991		0.999		0.9939		0.983	
Adjusted R^2		0.981		0.998		0.9861		0.961	
Predicted R^2		0.930		0.994		0.9670		0.843	
Adeq. Precision		29.589		128.15		33.0232		21.97	

* s Significant at ($p < 0.05$), A: Freezing time, B: Thawing time, C: Number of cycles Cor Total: Totals of all information corrected for the mean, Std. Dev.: Standard deviation, C.V.: Coefficient of variation.

Table 5
Validation of optimal conditions.

Particulars	Goal	Optimal value	Desirability	
		Predicted value	Experimental value	Error %
Oil yield (%)	Maximise	38.28 [a]	38.58 ± 1.09 [a]	0.55
Thermal diffusivity mm^2/s	in range	4.17 [a]	4.17 ± 0.05 [a]	0.11
TPC mg GAE/100 g	Maximise	80.64 [a]	80.42 ± 0.34 [a]	0.25
DPPH radical scavenging activity %	Maximise	49.63 [a]	49.88± 0.33 [a]	0.35

All values are conveyed as mean ± SD of three replicates.
a: Non significantly different.

3.4. Characterization of seeds

3.4.1. Morphological analysis

Freeze-thaw cycles are recognized for their ability to rupture cell wall of the seed due to thermal shock treatment (Phothiset & Charoenrein, 2014). SEM microstructure analysis in Fig. 2 shows a splitting of the seed coat that exposes the inner cotyledon, making it easier to extract the oil from FT-treated flaxseed. The FT treatments break down the polysaccharides and glycoproteins in the cell wall, making the wall simpler to break. The thermal shock caused by freeze-thaw cycles has a significant effect on the structural properties of flaxseeds, resulting in an enhanced oil yield. Freezing leads to the formation of ice crystals within the cells, while thawing results in expansion, thereby inducing mechanical stress on the cell wall, further exacerbating its structural breakdown. FT facilitated the degradation of polysaccharides, glycoproteins, and lignin in the cell wall, resulting in the cell membrane's physical disruption (Jiao et al., 2018). This ruptured cell structure leads to easier expulsion of oils and bioactive compounds from the FT-treated flaxseeds. Similar breakage in microstructures has been reported in perilla seeds, tiger nut seeds and cornmeal after FT pretreatment (Jiao et al., 2018; Lee et al., 2020; Zhang et al., 2024).

3.4.2. Engineering properties of seeds

The physical dimensions of flaxseeds, including length, width, and thickness, were found to be 5.05 mm, 2.5 mm, and 1.18 mm, respectively. It was found that there was a slight increase in the physical dimensions of FTO seeds, but they were not significant ($p > 0.05$) enough, whereas the seed volume of FTO seeds showed a significant difference ($p < 0.05$) from the UF (Table 6). The observed change might be due to the consequence of freezing injury that caused the cell wall to rupture during the FT process, increasing the surface area and volume of FTO seeds. The density parameter depends on mass per unit volume, and the change in expansion in volume results in a more porous seed structure, reducing the overall density of FTO seeds. Similarly, other factors, such as moisture levels, also affect the density of seeds (Singh & Meghwal, 2020). Furthermore, in terms of frictional properties, the FTO seeds exhibit significantly ($p < 0.05$) higher coefficient of friction and angle of repose due to increased porosity and irregular flaxseed surface that provide more point of contact with the surface in the FTO sample (Table 6). Similar physicochemical results of flaxseed were reported by (Patel et al., 2021). It was found that the broken flaxseed resulted in a significant increase ($p < 0.05$) in volume but a decrease in bulk density and tap density of FTO seeds. Density is one of the parameters that helps to design of seed to ensure efficient storage and flowability. The lower density parameters indicate that the seeds are loosened up and have less resistance to pressing, which contributes to enhanced pressing efficiency. This improved efficiency ultimately facilitates a more effective extraction process, leading to higher oil yield from flaxseed (Yang et al., 2024).

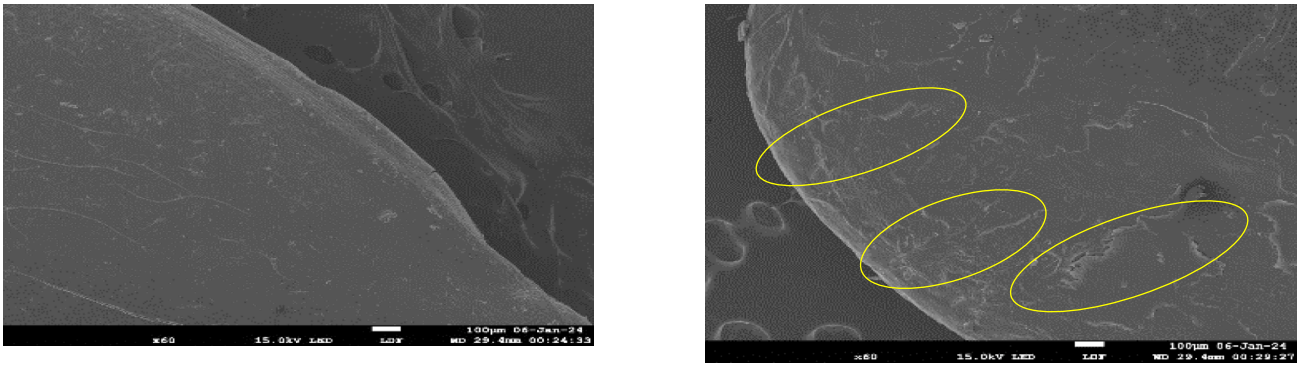
Table 6
Physical, gravimetrical and frictional properties of untreated flaxseed and Freeze-thaw optimized flaxseed.*

S·No	Parameters	UF	FTO seeds
1	Length (mm)	5.05 ± 0.21[b]	5.11 ± 0. 33 [a]
2	Width (mm)	2.5 ± 0.15 [a]	2.5 ± 0.15 [a]
3	Thickness (mm)	1.18 ± 0.06 [a]	1.29 ± 0.07 [a]
4	Seed surface (mm^2)	18.54 ± 0.26 [b]	19.66 ± 0.60 [a]
5	Seed volume (mm^3)	4.66 ± 0.05 [b]	5.13 ± 0.04 [a]
6	Sphericity (%)	0.9735 ± 0.12 [b]	1.0638 ± 0.33 [a]
7	Geometric mean (mm)	2.43 ± 0.45 [a]	2.50 ± 0.33 [a]
Gravimetric Properties			
1	Bulk density (Kg/m^3)	654.14 ± 3.43[a]	515.4 ± 2.3 [b]
2	True density (Kg/m^3)	1566.00 ± 3.38 [a]	1010.80 ± 2.76 [b]
3	Porosity (%)	49 ± 2.54 [b]	58 ± 0.06 [a]
4	Angle of repose °	14.40 ± 2.64 [b]	20.60 ± 2.36 [a]
Static coefficient of friction			
1	Plywood plate	0.885 ± 0.04 [b]	1.58815 ± 0.05 [a]
2	Glass	0.1137 ± 0.03 [b]	0.2915 ± 0.03 [a]
3	Aluminum	0.1519 ± 0.04 [b]	3.49 ± 0.03 [a]
4	GI sheet	0.1619 ± 0.05 [b]	0.30063 ± 0.04 [a]

UF: Untreated flaxseeds, FTO: Freeze-thaw optimized.
* All values are conveyed as mean ± SD of three replicates and significant at ($P < 0.05$) a and b statistically different, UF: Untreated flaxseed Oil, FTO: Freeze-thaw optimized flaxseed.

3.4.3. Thermal properties

Thermal diffusivity (mm^2/s) is associated with the velocity at which heat diffuses within a material, while thermal conductivity (W/mK)



(a) (b)

Fig. 2. Flaxseeds microstructure through scanning electron microscope (SEM) as affected by freeze-thaw (FT) pretreatment (a) Untreated flaxseeds (b) Freeze-Thaw optimized.

determines the rate at which heat can uniformly transfer to a food mass. In the study, the thermal diffusivity significantly increased ($p < 0.05$) from 1.08 mm^2/s to 4.17 mm^2/s and thermal conductivity increased ($p < 0.05$) from 66.09 to 75.48 W/mK in FTO seeds. Freezing injury creates voids and cracks within cells that might have contributed to rapid diffusion and increased thermal diffusivity. It might be possible that the FT process had broken the barriers that are resistant in the propagation pathway of heat, facilitating a higher flow of heat (Fig. 3). Moreover, the compact structure and high bulk density of UF typically create resistance in the propagation pathway of heat, which is reduced in the case of FTO seeds. Additionally, FT pretreatment causes volume expansion, resulting in a less obstructive path for heat conduction (Singh & Meghwal, 2020).

3.4.4. Oil yield

The FT process has tended to increase the oil yield by disrupting the cell wall. It was found that the oil yield drastically increased ($p < 0.05$) from 25.13 % to 38.28 % with the application of the FTO (freeze-thaw optimized) processing method, marking a significant 1.5 times (50 %) increase compared to the UF sample (Table 5). The enhancement is attributed to the mechanism involved in FT process, particularly due to the mechanical stress rupturing of cell membrane within cell walls, leading to the release oil from the vacuoles (Phothiset & Charoenrein, 2014). This cell wall rupturing is directly related to enhanced oil yield. During the FT process, the formation of large ice crystals exert pressure on the membranes, contributing to their rupture leading to breakdown of protein and carbohydrate bonds thus releasing entrapped oil (Yu et al., 2024). In addition, the freezing rate determines ice crystal size, the

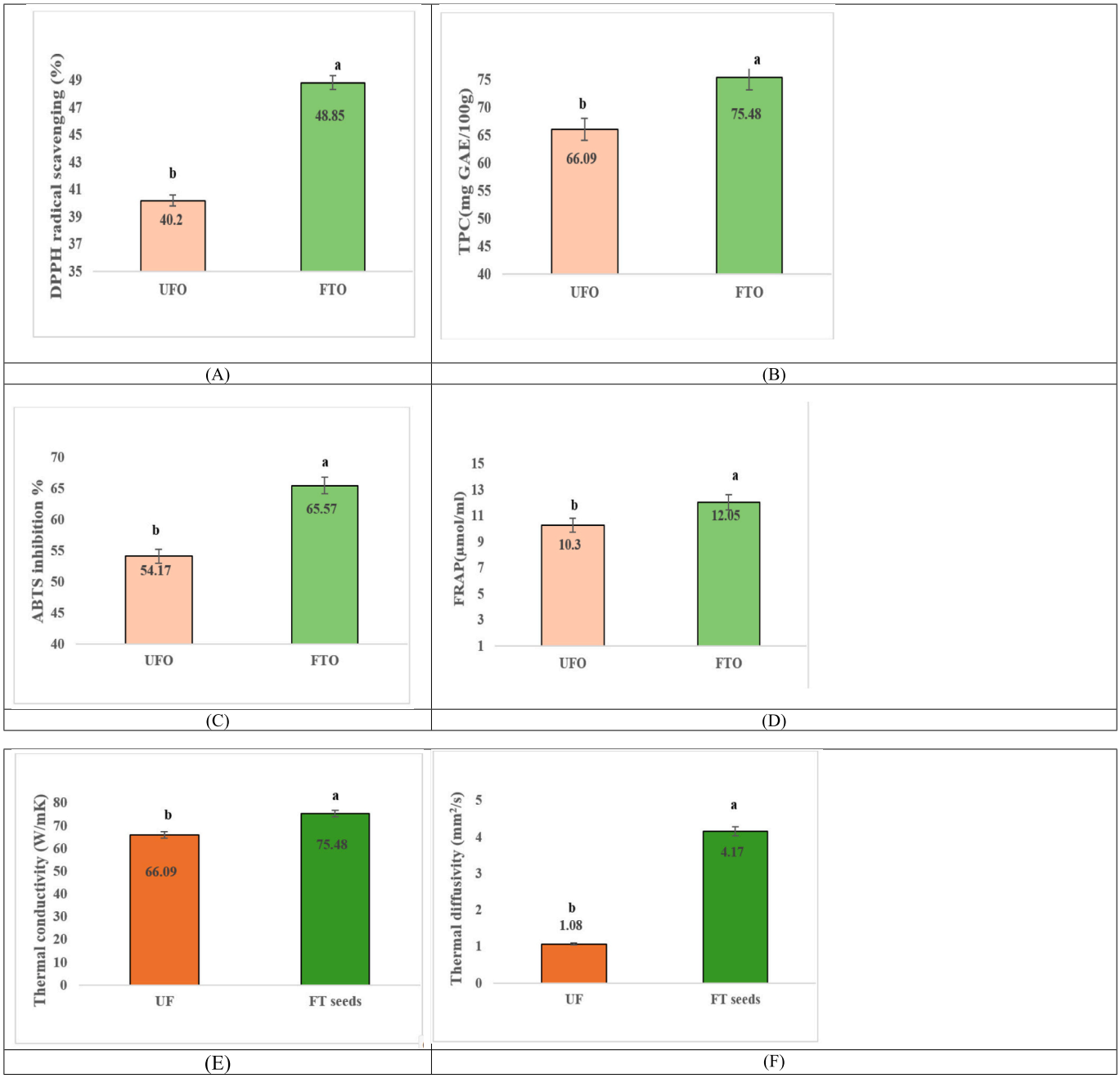


Fig. 3. (A) DPPH radical scavenging activity (%) (B) Total phenolic content (mg GAE/100 g) (C) ABTS inhibition (%) (d) FRAP (μmol/mL) of untreated flaxseed oil (UFO) and freeze-thaw optimized (FTO) oil: (E) Thermal conductivity(W/mK) (F) Thermal diffusivity (mm^2/s) of untreated flaxseed (UF) and freeze thaw optimized flaxseeds (FTO) flaxseeds.

extent of intra- and extra-cellular ice crystal formation, and the degree to which solutes are concentrated causing desiccation of cells (Zhao et al., 2015). Similarly earlier efforts to enhance oil yield from flaxseed lead to exploration of various pretreatments. For instance, Moknatjou et al. (2015) found that roasting increases the oil yield from 45 % to 53 %. However, this method is associated with the oxidation of PUFA as highlighted in their findings. Some other alternative pretreatments that aim to destroy cell walls using (cavitation) ultrasound, (cell wall digestion) enzymatic, and supercritical exhibited promising improvements in oil yield, with increases of 27 %, 26 %, and 30 %, respectively, compared to untreated cold pressed oil (Kulkarni et al., 2017). However, all of these treatments did not surpass the 50 % increase in oil yield obtained through FT pretreatment. In another study, microwave roasting pretreatment markedly increased the oil yield by 34.9 % but significantly diminished the antioxidant activity of oil (Suri et al., 2020).

3.5. Characterization of oil

3.5.1. Physicochemical properties

Physicochemical properties, including color, viscosity, the refractive index of UFO, and FTO oil (Table 7). The acid value (AV) and peroxide value (PV) serve as crucial parameters for evaluating the quality of edible oils. The acid value measures free fatty acids, which indicates the extent of hydrolytic rancidity, whereas the PV of oil is an empirical measure of oxidation products. The elevated AV and PV values suggest the onset of hydrolytic rancidity in oil and its derivatives. It was found that there were no significant differences ($p > 0.05$) in PV (2.0 EqO_2/Kg) and AV (1.47 mg/KOH/g) of FTO and UFO sample, falling within the CODEX limit that is <10 $meqO_2$/Kg and < 4 mg KOH/g respectively (Codex Alimentarius Commission, 2013). The FTO oil exhibited identical physicochemical properties to the UFO, indicating the absence of any degradation in oil quality. This effect might be due to optimal low-temperature conditions of the process, resulting in no oxidative and thermal changes in the chemical bonds within oil samples. The p-anisidine value is the amount of aldehydes in fats and oils formed during secondary oxidation in fats. The TOTOX value total oxidation value is calculated by adding twice the peroxide value (PV) to the anisidine value (AV). The p-anisidine and TOTOX values were found to be 0.50 and 4.5, exhibiting no significant difference ($p > 0.05$) from the UFO, indicating absence of secondary oxidation in FTO oil. Consequently, the color, refractive index, and viscosity have no statistical difference ($p > 0.05$), proving that FT treatment did not affect the physical quality of FO. The color of oil changes due to chemical reactions like maillard browning and caramelization, which are more likely to happen when oil seeds are exposed to heat during processing. The results obtained were in accordance with the results reported by Kasote et al. (2013). A similar trend was observed in perilla seed oil and tiger nut seed oil, where FT pretreatment resulted in no significant changes in oxidative and physical parameters (Lee et al., 2020; Zhang et al., 2024). Other pretreatments like roasting and microwave lead to thermal degradation and changes physicochemical properties of the oil, where a significant increase in PV and FFA was observed might be due to heat treatment (Moknatjou et al., 2015).

Table 7
Physicochemical properties and fatty acids of untreated flaxseed oil and FTO flaxseed oil.

Parameters		UFO	FTO oil
Physical properties			
Refractive Index (27 °C)		1.47342 ± 0.001[a]	1.47342 ± 0.001[a]
Color	R	1.3 ± 0.31[a]	1.3 ± 0.26[a]
	Y	49.33 ± 0.57[a]	49.31 ± 0.57[a]
	N	0.6 ± 0.34[a]	0.7 ± 0.23[a]
Viscosity (cp)		28 ± 0.01[a]	28 ± 0.01[a]
Chemical properties			
AV (mg KOH/g)		1.47 ± 0.05 [a]	1.47 ± 0.05 [a]
PV (meq O_2/Kg)		2.0 ± 0.04 [a]	2.0 ± 0.02 [a]
p- anisidine value		0.50 ± 0.02 [a]	0.5 ± 0.33 [a]
TOTOX value		4.5 ± 0.26 [a]	4.5 ± 0.40 [a]
Fatty Acid profile			
Palmitic acid (C16:0)		5.88 ± 0.03[a]	5.92 ± 0.04[a]
Stearic acid (C18:0)		5.73 ± 0.01[a]	5.97 ± 0.05[a]
Oleic acid (C18:1)		21.18 ± 0.42 [a]	21.2 ± 0.33[a]
Linoleic acid (C18:2)		13.24 ± 0.03[a]	13.39 ± 0.11[a]
Arachidic acid (C20:0)		0.19 ± 0.04[a]	0.19 ± 0.36[a]
Alpha linoleic acid (C18:3)		52.45 ± 0.24[a]	52.26 ± 0.27[a]
SFA		12.67 ± 0.09[a]	12.66 ± 0.09[a]
MUFA		21.37 ± 0.24[a]	21.38 ± 0.23[a]
PUFA		65.98 ± 0.34[a]	65.95 ± 0.14[a]

All values are conveyed as mean ± SD of three replicates. Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Poly unsaturated fatty acids, AV: acid value, PV: peroxide value, pAn: p Anisidine value, TOTOX: total oxidation, R- redness, Y- yellowness, N- Neutral, UFO: Untreated flaxseed oil, FTO: Freeze thaw optimized.

3.5.2. Fatty acid composition

The fatty acid profile was found to be dominant in alpha-linolenic acid (52 %), oleic acid (21 %), and linoleic acid (13 %), making it PUFA-rich, accounting for 65.98 % of the oil in UFO and 65.89 % in the FTO (Table 7). Interestingly, the fatty acid (S:M:P) Saturated: Monounsaturated: Polyunsaturated fatty acid ratio of both samples did not significantly ($p > 0.05$) differ from one another, potentially attributed to the low processing temperature and optimized process parameters, which prevented the deterioration of hydrocarbon bonds. These results were in accordance with earlier research on FO (Kasote et al., 2013). Similarly, Lee et al. (2020) observed no significant physicochemical changes in perilla seed oil were observed after FT treatment. However, contrary to that, alteration in the fatty acid composition with increased saturated and monounsaturated fatty acids was observed in tiger nut seed oil following FT treatment (Zhang et al., 2024). Other methods, such as roasting, significantly alter the fatty acid profile by decreasing alpha-linolenic acid and increasing SFAs (Sun et al., 2022). A similar increase in SFAs was reported by Choo et al. (2007) in pan heating and by Moknatjou et al. (2015) in microwave heating.

3.5.3. Antioxidant activity

The antioxidant capacity of oil indicates its stability and nutritional quality. Flaxseed oil is a rich source of bioactive compounds, specifically phenolics and lignans, that contribute to its antioxidant properties (Alaubody et al., 2024). However, these bioactive compounds, particularly polyphenols, are susceptible to heat and generally degraded during the pretreatment and processing stages. For instance, methods like microwave heating have been reported to negatively affect the DPPH activity with excessive microwave radiation on flaxseed oil (Szydłowska-Czerniak et al., 2020). While, in FT pretreatment, the antioxidant properties showed significant improvement ($p < 0.05$), with a 20 % increase in TPC. The DPPH radical scavenging activity of FTO oil increased by 24 %, while ABTS inhibition improved by 21 %, compared to UFO. Additionally, FRAP levels were enhanced by 17 %, rising from 10.33 μmol/mL in the UFO to 12.05 μmol/mL in FTO oil as compared to UFO (Fig. 3). This change could be attributed to two major factors. Firstly, cold-pressed extraction uses a mild treatment that avoids excessive chemicals and heat, which helps preserve antioxidants. Secondly, the impact of FT pretreatment, which caused damage to the inner cell wall, as evidenced by SEM images, might have promoted the leaching out of polyphenolics and other bioactive compounds from the cell. Moreover, FT pretreatment also damages insoluble constituents that bind these bioactive compounds to the cells, thereby releasing more antioxidant-rich compounds (Zhao et al., 2014). Similar trends have been reported in various plant-based sources, such as in corn kernels. FT pretreatment showed a significant increase in lutein from 2.23 to 16.39 μg/g, while zeaxanthin content increased from 4.66 to 36.3 μg/g (Jiao

et al., 2018). In another study, FT pretreatment also enhanced tocopherol and sterol extraction in tiger nut seed oil by 19.32 and 16.28 %, respectively (Zhang et al., 2024). Likewise, the phenolic and flavonoids increased by 56 % in carrot seed oil after the FT pretreatment (Ji et al., 2023).

4. Conclusion

The optimized freeze-thaw pretreatment has significantly ($p > 0.05$) increased oil yield and antioxidant properties due to the continuous thermal shock treatment, resulting in the enhanced release of oil and bioactive compounds. The major influential factors were the number of cycles, freezing time, and thawing time, identified through Taguchi prescreening analysis. The optimal extraction condition was found to be 6 h of freezing and 6 h of thawing for 4 cycles, resulting in 50 % significant increase ($p > 0.05$) in oil yield and a 24 % enhancement in bioactive compounds, leading to improved oxidative and nutritional stability. Additionally, the FT pretreatment retained the physicochemical properties as well as the fatty acid composition of the UFO, ensuring the retention of the omega-3 content of the oil. Overall, FT pretreatment is an efficient and eco-friendly technique with significant industrial potential, offering higher oil yield, improved nutritional quality, and enhanced stability of omega-3-rich oil.

Ethical approval

No ethical approval was required.

CRediT authorship contribution statement

Monika Chand: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Investigation, Formal analysis, Data curation, Conceptualization. **Rajni Chopra:** Writing – original draft, Visualization, Validation, Supervision, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Anupama Singh:** Visualization, Validation, Supervision, Formal analysis, Conceptualization. **Pramod K. Prabhakar:** Supervision, Software, Resources, Methodology, Formal analysis, Data curation, Conceptualization. **Aniket Kamboj:** Writing – review & editing, Writing – original draft, Software, Methodology, Investigation, Data curation, Conceptualization. **Priyanka Kumari Singh:** Visualization, Validation, Project administration, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2025.102328.

Data availability

Data will be made available on request.

References

Alaubody, N. M. T., Al-Mousawi, Z. A. H., Abdulwahid, A. B., & Khudair, Z. W. (2024). Physiological and histological effects of flaxseed oil on the liver and heart muscle. *Medical Journal of Babylon, 21*(3), 696–703. https://doi.org/10.4103/MJBL.MJBL_1603_23

AOAC. (2005). *Official methods of analysis of AOAC international* (18th ed.). AOAC International.

Benjakul, S., & Bauer, F. (2001). Biochemical and physicochemical changes in catfish (Silurus glanis Linne) muscle as influenced by different freeze–thaw cycles. *Food Chemistry, 72*(2), 207–217. https://doi.org/10.1016/S0308-8146(00)00222-3

Boerner, G., Brodkorb, S., Pufky-Heinrich, D., Franke, S., Polage, S., & Zang, M. (2020). *U.S. Patent No. 10,577,558*. Washington, DC: U.S. Patent and Trademark Office.

Bramante, F., Di Bari, V., Adams, G., Beaudoin, F., Waschatko, G., Jakobi, R., … Gray, D. A. (2025). Freeze-thaw stability of oilseed rape oleosome emulsions. *Journal of Food Engineering, 392*, Article 112471. https://doi.org/10.1016/j.jfoodeng.2025.112471

Choo, W. S., Birch, E. J., & Dufour, J. P. (2007). Physicochemical and stability characteristics of flaxseed oils during Pan-heating. *Journal of the American Oil Chemists' Society, 84*(8), 735–740. https://doi.org/10.1007/s11746-007-1096-7

Gauri, S. K., & Pal, S. (2017). Optimization of multi-response dynamic systems using multiple regression-based weighted signal-to-noise ratio. *International Journal of Industrial Engineering Computations, 161–178*. https://doi.org/10.5267/j.ijiec.2016.6.001

Ghosh, S., Bhattacharyya, D., & k., & Ghosh, M.. (2019). Comparative study of chemical characteristics, phytochemical contents and antioxidant activity of polar and non-polar solvent extracted flaxseed oil. *ACTA Pharmaceutica Sciencia, 57*(3), 65. https://doi.org/10.23893/1307-2080.APS.05718

Grand View Research. (2023). *Agriculture equipment market size, share & trends analysis report by product, by application, by region, and segment forecasts, 2023–2030*. Grand View Research: Retrieved from. https://www.grandviewresearch.com/industry-analysis/agriculture-equipment-market.

Ji, B., Lee, H., Jang, B.-K., Kim, Y.-J., Park, K., Jeong, H.-S., & Cho, J.-S. (2023). Extraction conditions to improve antioxidant and antibacterial activity of carrot seed oil. *Industrial Crops and Products, 202*, Article 116993. https://doi.org/10.1016/j.indcrop.2023.116993

Jiao, Y., Li, D., Chang, Y., & Xiao, Y. (2018). Effect of freeze-thaw pretreatment on extraction yield and antioxidant bioactivity of corn carotenoids (lutein and zeaxanthin). *Journal of Food Quality, 2018*, 1–8. https://doi.org/10.1155/2018/9843503

Kasote, D. M., Badhe, Y. S., & Hegde, M. V. (2013). Effect of mechanical press oil extraction processing on quality of linseed oil. *Industrial Crops and Products, 42*, 10–13. https://doi.org/10.1016/j.indcrop.2012.05.015

Kulkarni, N. G., Kar, J. R., & Singhal, R. S. (2017). Extraction of flaxseed oil: A comparative study of three-phase partitioning and supercritical carbon dioxide using response surface methodology. *Food and Bioprocess Technology, 10*(5), 940–948. https://doi.org/10.1007/s11947-017-1877-4

Lavenburg, V. M., Rosentrater, K. A., & Jung, S. (2021). Extraction methods of oils and phytochemicals from seeds and their environmental and economic impacts. *Processes, 9*(10), 1839. https://doi.org/10.3390/pr9101839

Lee, K.-Y., Rahman, M. S., Kim, A.-N., Son, Y., Gu, S., Lee, M.-H., … Choi, S.-G. (2020). Effect of freeze-thaw pretreatment on yield and quality of perilla seed oil. *LWT, 122*, Article 109026. https://doi.org/10.1016/j.lwt.2020.109026

Liu, Y., Wang, Y., Lv, W., Li, D., & Wang, L. (2021). Freeze-thaw and ultrasound pretreatment before microwave combined drying affects drying kinetics, cell structure and quality parameters of Platycodon grandiflorum. *Industrial Crops and Products, 164*, Article 113391. https://doi.org/10.1016/j.indcrop.2021.113391

Mane, S., Singh, A., & Taneja, N. K. (2024). Pretreatment optimization for microalgae oil yield enhancement and residual biomass characterization for sustainable biofuel feedstock production. *Biomass Conversion and Biorefinery*. https://doi.org/10.1007/s13399-024-05768-y

Melo, D., Álvarez-Ortí, M., Nunes, M. A., Costa, A. S. G., Machado, S., Alves, R. C., … Oliveira, M. B. P. P. (2021). Whole or defatted sesame seeds (Sesamum indicum L.)? The effect of cold pressing on oil and cake quality. *Foods, 10*(9), 2108. https://doi.org/10.3390/foods10092108

Moknatjou, R., Hajimahmoodi, M., Toliyat, T., Moghaddam, G., Sadeghpour, O., Monsef-Esfahani, H., & Shams-Ardekani, M. (2015). Effect of roasting on fatty acid profile of Brown and Yellow varieties of flaxseed (*Linum usitatissimum* L). *Tropical Journal of Pharmaceutical Research, 14*(1), 117. https://doi.org/10.4314/tjpr.v14i1.17

Nabil, M., & Khodadadi, J. M. (2013). Experimental determination of temperature-dependent thermal conductivity of solid eicosane-based nanostructure-enhanced phase change materials. *International Journal of Heat and Mass Transfer, 67*, 301–310. https://doi.org/10.1016/j.ijheatmasstransfer.2013.08.010

Patel, G., Naik, R. K., & Patel, K. K. (2021). *Studies on Some Physical and Engineering Properties of Linseed, 13*(3), 303–308.

Phinney, D. M., Frelka, J. C., Wickramasinghe, A., & Heldman, D. R. (2017). Effect of freezing rate and microwave thawing on texture and microstructural properties of potato (*Solanum tuberosum*). *Journal of Food Science, 82*(4), 933–938. https://doi.org/10.1111/1750-3841.13690

Phothiset, S., & Charoenrein, S. (2014). Effects of freezing and thawing on texture, microstructure and cell wall composition changes in papaya tissues: Effects of freezing and thawing on papaya tissues. *Journal of the Science of Food and Agriculture, 94*(2), 189–196. https://doi.org/10.1002/jsfa.6226

Prášil, I., & Zámečník, J.. (1998). The use of a conductivity measurement method for assessing freezing injury. *Environmental and Experimental Botany, 40*(1), 1–10. https://doi.org/10.1016/S0098-8472(98)00010-0

Rahman, M. H., Hossain, M. M., Rahman, S. M. E., Amin, M. R., & Oh, D.-H. (2015). Evaluation of physicochemical deterioration and lipid oxidation of beef muscle affected by freeze-thaw cycles. *Korean Journal for Food Science of Animal Resources, 35*(6), 772–782. https://doi.org/10.5851/kosfa.2015.35.6.772

Satpathy, S. K., Naik, S. N., & Jena, S. (2024). Moisture dependent physical, mechanical, and aerodynamic properties of *Simarouba glauca* seed: A potential tree born oilseed. *Journal of Food Process Engineering, 47*(1), Article e14513. https://doi.org/10.1111/jfpe.14513

Shafizah, I. N., Irmawati, R., Omar, H., Yahaya, M., & Aina, A. A. (2022). Removal of free fatty acid (FFA) in crude palm oil (CPO) using potassium oxide/dolomite as an adsorbent: Optimization by Taguchi method. *Food Chemistry, 373*, 131668. https://doi.org/10.1016/j.foodchem.2021.131668

Singh, H., & Meghwal, M. (2020). Physical and thermal properties of various ajwain *(Trachyspermum ammi L.)* seed varieties as a function of moisture content. *Journal of Food Process Engineering, 43*(2), Article e13310. https://doi.org/10.1111/jfpe.13310

Singh, P. K., Chopra, R., Garg, M., Chauhan, K., & Agarwal, A. (2024). Stability of perilla seed oil based PUFA-rich structured lipids using enzymatic interesterification: A thermo-oxidative kinetic study. *Industrial Crops and Products, 209*, Article 118029. https://doi.org/10.1016/j.indcrop.2024.118029

Sun, X., Zhang, B., Han, J., Wei, C., & Liu, W. (2022). Effect of roasting temperature and time on volatile compounds, total tocopherols, and fatty acids of flaxseed oil. *Journal of Food Science, 87*(4), 1624–1638. https://doi.org/10.1111/1750-3841.16073

Suri, K., Singh, B., Kaur, A., Yadav, M. P., & Singh, N. (2020). Influence of microwave roasting on chemical composition, oxidative stability and fatty acid composition of flaxseed (Linum usitatissimum L.) oil. *Food Chemistry, 326*, Article 126974. https://doi.org/10.1016/j.foodchem.2020.126974

Szydłowska-Czerniak, A., Tymczewska, A., Momot, M., & Włodarczyk, K. (2020). Optimization of the microwave treatment of linseed for cold-pressing linseed oil—Changes in its chemical and sensory qualities. *LWT, 126*, Article 109317. https://doi.org/10.1016/j.lwt.2020.109317

Tu, D., Ou, Y., Zheng, Y., Zhang, Y., Zheng, B., & Zeng, H. (2021). Effects of freeze-thaw treatment and pullulanase debranching on the structural properties and digestibility of lotus seed starch-glycerin monostearin complexes. *International Journal of Biological Macromolecules, 177*, 447–454. https://doi.org/10.1016/j.ijbiomac.2021.02.168

Wang, L., Dong, B., Yang, T., Zhang, A., Hu, X., Wang, Z., Chang, G., & Chen, G. (2022). Dietary linseed oil affects the polyunsaturated fatty acid and transcriptome profiles in the livers and breast muscles of ducks. *Frontiers in Nutrition, 9*, 1030712. https://doi.org/10.3389/fnut.2022.1030712

Yang, L., Cui, B., Chen, H., Wu, J., Xu, Z., Xiao, X., Wang, Z., Song, S., & Zhang, Y. (2024). Research on seeds compression-structural damage and castor oil extraction mechanism with in situ observation. *Journal of Food Process Engineering, 47*(8), Article e14708. https://doi.org/10.1111/jfpe.14708

Zhang, F., Lin, P.-X., Xia, P.-X., Di, H.-M., Zhang, J.-Q., Wang, Z.-H., Li, Z.-Q., Huang, S.-Y., Li, H.-X., & Sun, B. (2021). The effect of different thawing methods on the health-promoting compounds and antioxidant capacity in frozen baby mustard. *RSC Advances, 11*(17), 9856–9864. https://doi.org/10.1039/D1RA00610J

Zhang, Z., Xie, X., Jia, H., Le, W., & Xiang, P. (2024). Effect of freeze-thaw treatment on the yield and quality of tiger nut oil. *Food Chemistry: X, 23*, Article 101733. https://doi.org/10.1016/j.fochx.2024.101733

Zhao, J., Dong, F., Li, Y., Kong, B., & Liu, Q. (2015). Effect of freeze–thaw cycles on the emulsion activity and structural characteristics of soy protein isolate. *Process Biochemistry, 50*(10), 1607–1613. https://doi.org/10.1016/j.procbio.2015.06.021

Zhao, S., Baik, O.-D., Choi, Y. J., & Kim, S.-M. (2014). Pretreatments for the efficient extraction of bioactive compounds from plant-based biomaterials. *Critical Reviews in Food Science and Nutrition, 54*(10), 1283–1297. https://doi.org/10.1080/10408398.2011.632698