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Enrichment of Essential Omega-3 Fatty Acids from Flaxseed Oil Using Urea Complexation and Cold Crystallization

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ABSTRACT

Flaxseed (*Linum usitatissimum*) oil is a rich botanical source of EFAs, specifically α-linolenic acid (ALA, ω-3) and linoleic acid (LA, ω-6), known for their health benefits. This study comparatively evaluated urea complexation and low-temperature crystallization (LTC) for enriching essential fatty acids (EFAs) from flaxseed oil. Fatty acid profiles were determined by gas chromatography, and recovery yields assessed process efficiency. The crude oil contained 54.13% ALA, 15.02% LA, 19.36% oleic acid, and 10.5% saturated fatty acids (SFAs), with total EFAs comprising 69.15% of the profile. Urea complexation produced a non-urea-complexed fraction (NUCF) highly enriched in EFAs (63.0% ALA, 17.0% LA) and with saturated fatty acids (SFAs) reduced to 1.9%. However, this method had a low overall recovery yield of only 37.4%. In contrast, LTC yielded a liquid fraction with a slightly lower enrichment (55.2% ALA, 15.25% LA) and SFAs at 6.62%, but it achieved a significantly higher overall recovery yield of 96%. This work is the first direct comparative evaluation of these methods for flaxseed oil, highlighting a key trade-off between compositional purity and material recovery. The findings provide guidance for selecting an enrichment strategy aligned with specific industrial or nutraceutical goals.

1- INTRODUCTION

The functional food industry has experienced unprecedented growth driven by increasing consumer awareness of the relationship between diet and health outcomes (Gul et al., 2016). Essential fatty acids (EFAs), specifically the polyunsaturated fatty acids (PUFAs) α-linolenic acid (ALA, ω-3) and linoleic acid (LA, ω-6), are vital components of human nutrition that cannot be synthesized *de novo* and therefore require dietary intake to maintain physiological functions, including cardiovascular health, neurodevelopment, immune regulation, and cell membrane integrity (De Carvalho & Caramujo, 2018). A central challenge in delivering health-promoting PUFAs lies in identifying and efficiently extracting suitable sources. Marine lipids have traditionally dominated the omega-3 market, yet sustainability concerns, cost, oxidative instability, and dietary preferences have driven growing demand for plant-based alternatives. Among terrestrial oilseeds, flax (*Linum usitatissimum* L.) represents one of nature's richest sources of ALA and emerges as a particularly compelling feedstock for omega-3 enrichment (Safdar et al., 2020). The flax plant is an annual herb with slender stems (0.5–1.2 m), lanceolate leaves, and blue or white flowers that form seed capsules containing 6–10 seeds. The seeds are small (3–6 mm long), oval-shaped, and oil-rich, with a glossy surface that ranges from golden-yellow to dark brown. Nutritionally, flaxseed comprises approximately 41% oil, 28% dietary fiber, 20% protein, and 6% carbohydrates. Beyond its lipid content, flaxseed contains a diverse array of bioactive secondary metabolites that enhance its functional food potential, including lignans, phenolic compounds, mucilaginous polysaccharides, and cyanogenic glycosides (Muir & Westcott, 2003; Peterson et al., 2010). These secondary metabolites contribute additional health-promoting properties including antioxidant, phytoestrogenic, and prebiotic activities that complement the omega-3 fatty acid benefits. The lipid fraction exhibits a distinctive fatty acid profile particularly valuable for functional food development: ~70% PUFAs (including ~12–19% LA and ~40–60% ALA), ~12–19% monounsaturated fatty acids (MUFAs), and only ~8–11% saturated fatty acids (SFAs). Although flaxseed oil possesses an inherently favorable fatty acid profile, it also contains nutritionally less desirable SFAs and MUFAs that dilute its omega-3 density. To reach therapeutic or functional fortification levels in foods, high quantities of unrefined oil may be required, posing challenges in sensory quality, oxidative stability, and formulation economics. Therefore, methods that can selectively concentrate PUFAs, particularly ALA, without compromising lipid integrity are of significant interest.

Several PUFA enrichment technologies have been developed, including supercritical CO_2 extraction, molecular distillation, and enzymatic modification. However, these methods often involve high operating costs, technical complexity, or the risk of degrading sensitive fatty acids (Rollin et al., 2023). In response, interest has grown in simpler and more selective approaches such as urea complexation and low-temperature crystallization (LTC), both of which offer milder processing conditions and scalable potential.

Urea complexation relies on the unique ability of urea to form crystalline inclusion complexes with linear, SFAs, and MUFAs. The kinked structures of polyunsaturated species prevent their incorporation into the urea lattice, allowing for PUFA enrichment in the filtrate (Wanasundara & Shahidi, 1999). Conversely, LTC operates through phase separation induced by temperature: saturated and monounsaturated fatty acid methyl esters (FAMEs) crystallize upon cooling, while PUFAs, owing to their lower melting points and molecular disorder, remain in solution (Bodkowski et al., 2020). Both techniques leverage structural features of fatty acids to achieve separation, but their

relative efficacy in ALA-dominant oils remains unclear.

Importantly, most prior investigations have centered on long-chain marine omega-3 PUFAs, such as EPA and DHA, which differ in molecular characteristics from ALA (Eskandari et al., 2024; Ghasemian et al., 2015; Kosasih et al., 2023; Maruyama et al., 2022; Soleimanian et al., 2015; Zhang et al., 2017). Whether urea complexation and LTC can achieve comparable selectivity and enrichment performance with short-chain omega-3s like ALA, particularly in flaxseed oil matrices, warrants focused study. To the best of our knowledge, this research represents the first systematic comparative evaluation of urea complexation and low-temperature crystallization for the enrichment of omega fatty acids from flaxseed oil. Using gas chromatography to analyze fatty acid profiles, we assess each method's capacity to selectively concentrate ALA, minimize SFA and MUFA content, and yield fractions suitable for use in functional foods and nutraceutical formulations. By illuminating the operational strengths and limitations of both strategies in a plant-based lipid context, this study contributes to the development of scalable technologies for next-generation omega-3-enriched ingredients that meet contemporary demands for sustainability, functionality, and health impact.

2- Materials and methods

2.1. Preparation of flaxseed oil

Premium quality flaxseeds (*L. usitatissimum*) were sourced from a certified organic supplier located in Amol, Mazandaran Province, Iran. The seeds were characterized by their typical morphological features: small, flat, oval-shaped structure (average dimensions 4.2 ± 0.3 mm length, 2.4 ± 0.2 mm width), glossy brown surface, and uniform size distribution indicating optimal maturity. Prior to oil extraction, the seeds underwent thorough cleaning to remove foreign materials, damaged seeds, and plant debris, followed by air-drying at ambient temperature (20-25°C) for 48 hours to achieve optimal moisture content (<10%) for efficient extraction. Cold-press extraction was performed using a laboratory-scale mechanical screw press (AG-OPR 001, Iran) operated at ambient temperature to preserve the integrity of thermolabile compounds including PUFAs. The extraction process was conducted under controlled conditions with pressing temperature maintained below 40°C to minimize oxidative degradation and preserve the natural antioxidant profile of the oil. The freshly extracted oil exhibited the characteristic golden-amber color and nutty aroma typical of high-quality flaxseed oil. The crude oil was subsequently filtered through Whatman No. 4 filter paper to remove particulate matter and residual seed fragments, then stored in amber glass bottles at 4°C to prevent photo-oxidation and maintain oil quality until further analysis and processing.

2.2. Gas chromatographic conditions

Fatty acid methyl esters (FAMEs) derived from flaxseed oil and its processed fractions were analyzed using a gas chromatograph (GC-FID) (Agilent 7390A) equipped with a DB-WAX capillary column (60 m × 0.25 mm i.d., 0.25 µm film thickness; polyethylene glycol stationary phase). The oven temperature was initially held at 170 °C for 5 minutes, followed by a programmed increase to 220 °C at a rate of 1 °C/min. The injector and flame ionization detector (FID) temperatures were both maintained at 220 °C. Nitrogen was used as the carrier gas at a constant flow rate of 1.0 mL/min. Sample injection was performed in split mode, and peak identification was accomplished by comparing the retention times of sample components with those of standard FAMEs analyzed under identical conditions. Quantification was based on relative peak area percentages obtained from the FID signal without the application of individual response correction factors. The method enabled precise resolution of major SFAs, MUFAs, and PUFAs, including palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic, and α-linolenic acid, across crude and processed flaxseed oil samples.

2.3. Fatty acid profiling in crude flaxseed oil

Comprehensive fatty acid profiling of flaxseed oil was conducted following the Iranian National Standards Organization protocol (INSO 13126-2). For methylation, precisely 100 mg of flaxseed oil was transferred to a 15 mL screw-cap glass tube, followed by the addition of 2 mL of *n*-hexane and 200 µL of freshly prepared 2 M methanolic potassium hydroxide solution. The methylation reaction was conducted at room temperature (22 ± 2°C) with gentle agitation for 30 minutes to ensure complete transesterification of triglycerides to fatty acid methyl esters (FAMEs). This mild alkaline methylation method was selected to prevent artifacts formation and preserve the integrity of PUFAs, particularly alpha-linolenic acid which is susceptible to thermal and chemical degradation. Following the methylation reaction, the mixture was neutralized and dried over anhydrous sodium sulfate to remove residual moisture that could interfere with chromatographic analysis. The organic phase containing the FAMEs was carefully separated and concentrated to achieve appropriate concentration for gas chromatographic injection. The resulting FAMEs were subsequently analyzed using the gas chromatographic method described in Section 2.2, with peak identification accomplished through comparison of retention times with authenticated FAME standards and confirmation using mass spectrometric data when necessary.

2.4. Preparation of free fatty acids

The extraction of free fatty acids (FFAs) from flaxseed oil was performed using a modified saponification method based on Vannasundara et al. (Wanasundara & Shahidi, 1999). Flaxseed oil (25.0 g) was refluxed with potassium hydroxide (5.75 g), distilled water (11 mL), and ethanol (66 mL) at 80°C under nitrogen atmosphere for 1 h to ensure complete saponification. After cooling to room temperature, distilled water (50 mL) was added to the reaction mixture. The non-saponifiable fraction was removed by extraction with hexane (2 × 100 mL), and the combined organic extracts were discarded. The aqueous layer was acidified to pH 1.0 using 3 N hydrochloric acid (pH monitored with a calibrated pH meter) to protonate the fatty acid salts. The liberated free fatty acids were extracted with hexane (3 × 50 mL) using a separatory funnel. The combined hexane extracts were dried over anhydrous sodium sulfate for 30 min, filtered through cotton, and concentrated using a rotary evaporator at 40°C under reduced pressure. The yield was determined and reported in the Results section. The purified free fatty acids were stored at −70°C until further use.

2.5. Enrichment of essential fatty acids via urea complexation

The enrichment of essential fatty acids from hydrolyzed flaxseed oil was conducted using urea complexation, based on established procedures reported in the literature (Wanasundara & Shahidi, 1999), with slight modifications. FFAs (10.0 g), obtained from section 2.4, were combined with 35 mL of a urea solution (20% w/v) prepared by dissolving urea in 95% ethanol, in a round-bottom flask equipped with a magnetic stirrer. The mixture was heated to 60°C with constant stirring until complete dissolution was achieved, forming a clear, homogeneous solution. The solution was allowed to cool to -4°C for 12 h under static conditions. The crystalline fraction (UCF) was separated from the non-complexed fraction (NUCF) by vacuum filtration using a Büchner funnel (porosity 3) pre-cooled to -4°C. The filtrate containing NUCF was diluted with an equal volume of distilled water and acidified to pH 4.0 ± 0.2 using 6 N hydrochloric acid (pH monitored with calibrated pH meter). The acidified solution was extracted with hexane in a separatory funnel. The combined hexane extracts were washed with distilled water, and dried with anhydrous sodium sulfate, filtered, and concentrated using a rotary evaporator at 40°C under reduced pressure. Fatty acids from the UCF crystals were recovered by dissolving the solid in distilled water, acidifying to pH 4.0 with 6 N HCl, and extracting with hexane following the same workup procedure as described for NUCF. The fatty acid

compositions of both UCF and NUCF fractions were determined by gas chromatography as described in section 2.2. The recovery yield (%) of each fraction was calculated using the following equation:

Recovery yield (%) =
Mass of recovered fraction (g) /
Initial mass of FFAs (g) ×100

2.6. Enrichment of fatty acids via low-temperature crystallization

Selective crystallization was employed to further concentrate omega-3 and omega-6 fatty acids from flaxseed-derived free fatty acids, following a modified protocol adapted from previously reported methods in the literature (Homayooni et al., 2014). Free fatty acids (11.0 g) from section 2.4 were completely dissolved in absolute ethanol (200 mL) in a jacketed crystallization vessel equipped with a magnetic stirrer and temperature probe. The solution was cooled using a programmable cooling bath at a controlled rate of 2°C/h to –4°C for 24 h to promote selective crystallization of saturated and less unsaturated fatty acids. The crystalline solid phase was separated from the liquid supernatant by vacuum filtration through a pre-cooled Büchner funnel at –4°C. The crystals were washed with ice-cold absolute ethanol (2 × 5 mL) to remove residual non-crystallized components. The liquid fractions (enriched in omega-3 and omega-6 fatty acids) was concentrated using a rotary evaporator at 40–45°C under reduced pressure. Complete solvent removal was confirmed by constant mass upon repeated evaporation.

The masses of both solid and liquid fractions were recorded after drying. The fatty acid compositions of each fraction were determined as described in section 2.2.

The recovery yield (%) of each fraction was calculated using the following equation:

Recovery yield (%) =
Mass of recovered fraction (g)/
Initial mass of FFAs (g)

2.7. Materials and equipment

All reagents and solvents were of analytical or chromatographic grade and were procured from certified suppliers, including Merck (Germany) and Sigma-Aldrich (USA).

3- Results and discussion

3.1. Fatty acids composition of crude flaxseed oil

Gas chromatography (GC) analysis enabled the identification and quantification of five major fatty acids in flaxseed oil: palmitic acid, stearic acid, oleic acid, linoleic acid, and α-linolenic acid. The relative abundance of each fatty acid was determined to be 5.4% for palmitic acid, 5.1% for stearic acid, 19.36% for oleic acid, 15.02% for LA, and 54.13% for ALA (Fig. 1). Representative gas chromatograms for the crude flaxseed oil is provided (Fig. S1) to illustrate peak resolution and fatty acid identification.

Analysis of the lipid profile revealed that total SFAs, primarily comprising palmitic and stearic acids, constituted 10.5% of the total fatty acid content. Conversely, UFAs predominated, accounting for 88.51%. Within the UFA fraction, MUFAs, represented by oleic acid, comprised 19.36%, while PUFAs, encompassing linoleic and α-linolenic acids, amounted to 69.15%. These values are consistent with the typical fatty acid composition reported for flaxseed oil (Khourang et al., 2011).

The SFA to UFA ratio was determined to be 0.12, underscoring the significant prevalence of unsaturated over SFAs. Further analysis indicated an SFA to PUFA ratio of 0.15 and a PUFA to MUFA ratio of approximately 3.57, collectively highlighting the substantial enrichment of polyunsaturated components, notably essential fatty acids. Crucially, the combined content of essential PUFAs (ALA and LA) totaled 69.15% (Table 1). The ω-3 to ω-6 ratio was approximately 3.6:1. This ratio is considered nutritionally advantageous given the typical ω-6 dominance in contemporary diets, which is often associated with various chronic conditions (Simopoulos, 2016a). The favorable ω-3/ω-6 balance observed in flaxseed

oil suggests a potential role in mitigating the risk of cardiovascular, neurodegenerative, and inflammatory diseases. Among the UFAs, oleic acid (MUFA) accounted for 19.36%, a component recognized for its contributions to cardiovascular health through its purported support of high-density lipoprotein cholesterol levels and maintenance of membrane fluidity. The comparatively low levels of SFA align with the general fatty acid composition characteristic of plant-based oils, particularly those derived from oilseeds, thereby further enhancing the perceived health benefits of flaxseed oil.

3.2. Fatty acid composition of flaxseed oil processed by urea complexation

Urea complexation has emerged as a highly effective and selective method for the enrichment of PUFAs, particularly omega-3 fatty acids, from complex lipid matrices. This technique capitalizes on the unique ability of urea to form crystalline inclusion complexes preferentially with linear-chain SFAs and MUFA In contrast, polyunsaturated species, due to the presence of multiple cis double bonds that introduce kinks into the hydrocarbon chain, are sterically excluded from the urea lattice. This molecular discrimination facilitates a phase separation, wherein SFAs and MUFAs are retained in the precipitated urea-complexed fraction (UCF), while PUFAs are concentrated in the filtrate, referred to as the non-urea-complexed fraction (NUCF). In this study, the urea complexation method was employed to fractionate flaxseed oil fatty acid, resulting in a marked redistribution of fatty acid classes between the urea complexed and non-complexed fractions, as confirmed by gas chromatographic analysis (Fig. S2).

The UCF retained 7.0% palmitic acid, 7.0% stearic acid, 20.0% oleic acid, 13.0% LA, and 49.0% ALA. These values correspond to 14.0% SFA, 20.0% MUFA, and 62.0% PUFA, yielding calculated lipid ratios of SFA:UFA = 0.17, SFA:PUFA = 0.23, and PUFA:MUFA = 3.1 (Table 1). While this fraction demonstrated moderate PUFA retention, it still contained a substantial portion of saturated and monounsaturated species, reflecting incomplete exclusion during crystallization.

Strikingly, the NUCF exhibited a pronounced enrichment of PUFAs, particularly EFAs. The fatty acid profile included only 0.9% palmitic acid, 1.0% stearic acid, 17.0% oleic acid, 17.0% LA, and a substantial 63.0% ALA (Fig. 2). These values translate into 1.9% SFA, 17.0% MUFA, and 80.0% PUFA, with highly favorable ratios: SFA:UFA = 0.02, SFA:PUFA = 0.024, and PUFA:MUFA = 4.7 (Table 1). Notably, the total EFA content, defined here as the combined proportion of LA and ALA, rose from 71% in the crude oil to 80% in the NUCF. This represents not only a quantitative enrichment but also a qualitative optimization of the oil's nutritional profile. The elevation of ALA from 54% to 63% is particularly significant, given its established roles in modulating inflammation, supporting cardiovascular function, and maintaining neural health. A parallel increase in LA (from 15% to 17%) enhances the lipid's dietary completeness, given the indispensable roles of both omega-3 and omega-6 PUFAs in human metabolism.

Regarding the processing yields, the NUCF was recovered at 23% of the total mass, representing a relatively low yield, but justified by the exceptional compositional purity. The UCF accounted for 14.4% of the mass, while the remaining material was lost during processing and filtration. Although this highlights the inherent trade-off between selectivity and yield, the resulting NUCF fraction offers a uniquely enriched lipid profile suitable for high-value applications.

These findings provide compelling evidence that urea complexation is a powerful tool for refining plant-based lipids into PUFA-rich formulations with elevated functional and therapeutic value. The selective exclusion of SFAs and MUFAs, enabled by precise molecular interactions during crystallization, affords a streamlined method for obtaining lipid fractions tailored to nutritional, pharmaceutical, and nutraceutical applications. Importantly, the substantial increase in EFAs confirms the

technique's applicability for the targeted development of omega-3 and omega-6 enriched ingredients, meeting the growing demand for plant-derived functional lipids.

The results herein align with previous work by Wanasundara and Shahidi (Wanasundara & Shahidi, 1999), while advancing its application within the context of modern dietary science and the growing demand for plant-based functional lipids.

In summary, the application of urea complexation to flaxseed oil fatty acid produced a lipid fraction with a substantially improved PUFA profile, enhanced essential fatty acid content, and optimized health-promoting ratios. While the method entails a compromise in mass recovery, its precision and efficacy render it a valuable tool for formulating next-generation lipid ingredients aligned with precision nutrition, preventive healthcare, and sustainable food innovation.

3.3. Fatty acid composition of flaxseed oil processed by recrystallization

Low-temperature crystallization (LTC) is a strategic solvent-assisted technique that leverages the intrinsic differences in melting points and solubility among lipid classes to fractionate complex fatty acid mixtures with high selectivity and minimal structural disruption. By cooling a lipid solution to sub-zero temperatures, SFAs owing to their differential solubility and higher crystallization temperatures, precipitate into a solid crystalline phase. In contrast, unsaturated fatty acids, particularly those with multiple *cis* double bonds such as LA and ALA, remain solubilized due to their greater conformational disorder and reduced lattice compatibility. This temperature-dependent phase behavior allows for the efficient separation and enrichment of bioactive unsaturated lipids without the need for chemical derivatization, making LTC an ideal tool for nutritional lipid enhancement.

In this study, LTC was employed to fractionate fatty acids derived from flaxseed oil. This process yielded two distinct phases: a dense solid crystalline fraction and a residual liquid phase. Gas chromatographic analysis was conducted to elucidate the fatty acid distribution in each fraction (Fig. S3).

The crystalline fraction was heavily enriched in saturated species, comprising 24.0% palmitic acid and 63.0% stearic acid, for a combined SFA content of 87.0%. Unsaturated fatty acids were minimally represented, with 2.0% oleic acid, 1.0% LA, and 5.0% ALA, totaling 2.0% MUFA and 6.0% PUFA (Fig. 3). The resulting lipid ratios—SFA:UFA = 10.88, SFA:PUFA = 14.5, and PUFA:MUFA = 3.0 (Table 1), highlight the pronounced selectivity of LTC in concentrating saturated molecules within the solid matrix, effectively depleting valuable unsaturated species.

In stark contrast, the liquid fraction exhibited a lipid profile markedly enriched in unsaturated fatty acids. SFAs were reduced to a total of 6.62% (4.7% palmitic and 1.92% stearic acid), while MUFAs and PUFAs were substantially elevated, accounting for 20.9% and 70.45%, respectively. The PUFA fraction was composed of 15.25% LA and an impressive 55.2% ALA. Overall, the unsaturated fatty acid content reached 91.35%, with remarkably favorable lipid ratios: SFA:UFA = 0.072, SFA:PUFA = 0.094, and PUFA:MUFA = 3.37 (Table 1). These results underscore the capability of LTC to produce a highly refined, PUFA-rich lipid fraction that is exceptionally low in saturated fats.

Notably, the ALA concentration in the liquid phase rose from approximately 54% in crude flaxseed oil to 55.2% post-crystallization, an enrichment achieved without oxidative stress or chemical intervention. The retention of LA at 15.25% further contributed to a balanced omega-3 to omega-6 ratio of 3.62:1, exceeding typical dietary ratios and aligning with recommendations for anti-inflammatory lipid intake. This compositional shift not only amplifies the nutritional profile of flaxseed oil but also enhances its potential functionality in preventing chronic disease conditions linked to lipid imbalance.

From a processing standpoint, the liquid fraction from LTC was recovered at 75% of the

total mass, representing a high yield for a fractionation process. The solid fraction accounted for 21% of the mass. The combined recovery of 96% indicates minimal material loss during the crystallization process. This efficient recovery, particularly of the liquid fraction with its desirable fatty acid profile, highlights the efficacy of LTC for obtaining high-value lipid fractions with practical applicability.

The compositional trends observed here strongly corroborate earlier reports by Homayooni et al. (Homayooni et al., 2014), reaffirming the robustness and reproducibility of LTC as a selective fractionation tool for bioactive fatty acids.

In summary, LTC presents a practical, chemically benign, and scalable approach for tailoring flaxseed oil toward higher nutritional functionality. By selectively depleting SFAs and enriching omega-3 and omega-6 fatty acids, it transforms crude oil into a bioactive lipid fraction aligned with contemporary dietary recommendations. With its high PUFA recovery (75%), strong process simplicity, and environmental compatibility, LTC emerges not merely as a separation method, but as a precision lipid engineering strategy suitable for developing next-generation functional foods, therapeutic lipids, and nutraceutical formulations.

The comparative analysis of fatty acid profiles between crude flaxseed oil and its processed fractions obtained via urea complexation and LTC underscores the strategic potential of these techniques in enriching PUFAs. While both methods aim to enhance the nutritional value of flaxseed oil by concentrating EFAs, they do so through fundamentally distinct physicochemical mechanisms. This divergence yields complementary insights into their selectivity, recovery efficiency, scalability, and suitability for various industrial and health-oriented applications.

Crude flaxseed oil, already notable for its favorable lipid composition, contained 54.13% ALA, 15.02% LA, and 19.36% oleic acid, alongside 10.5% total SFAs, composed of nearly equal parts palmitic and stearic acids. Although inherently rich in omega-3 PUFAs, the presence of SFAs and non-essential MUFAs tempers the oil's nutritional density with respect to EFAs. Accordingly, enrichment strategies are justified to elevate its biofunctional value and meet the demands of precision nutrition.

Urea complexation, grounded in the formation of crystalline inclusion complexes between urea and linear-chain saturated and monounsaturated FAMEs, offers a molecular-level separation strategy (Eskandari et al., 2024). Kinked PUFA chains, such as ALA and LA, are sterically excluded and retained in the NUCF. The results are striking: ALA content rose to 63%, LA to 17%, while SFAs were reduced to 1.9% (0.9% palmitic and 1.0% stearic). The total PUFA content reached 80%, significantly higher than the 69.15% present in the crude oil. These improvements reflect the exceptional selectivity of the method. However, the NUCF yield was limited to 23%, and the UCF constituted 14.4% of the total mass, highlighting a trade-off between compositional purity and material efficiency. Furthermore, the use of hexane, excess urea introduces concerns regarding environmental sustainability, process safety, and cost-effectiveness. As such, this method is best suited for high-value formulations such as medical-grade omega-3 supplements where purity supersedes yield and process complexity is justifiable. In contrast, LTC employs a phase-based separation governed by differential solubility and melting behavior. By leveraging the propensity of SFAs to crystallize at sub-zero temperatures, LTC enables their removal into a solid fraction, leaving PUFAs enriched in the liquid phase (Zhang et al., 2017). The solid fraction, recovered at a 21% yield, was dominated by SFAs, while the liquid fraction, constituting 75% of the initial mass, contained 55.2% ALA and 15.25% LA, amounting to a total PUFA content of 70.45%. Although this represents a slightly lower PUFA enrichment compared to the NUCF, the remarkably higher recovery efficiency and operational simplicity make

LTC highly attractive for large-scale applications. Notably, SFA content in the liquid fraction obtained via LTC decreased to 6.62%, representing a 37% reduction relative to the crude flaxseed oil. Concurrently, the ω-3 to ω-6 fatty acid ratio improved to approximately 3.62, aligning with the optimal balance associated with anti-inflammatory activity and cardiometabolic protection (Sheppard & Cheatham, 2018; Simopoulos, 2016b). These findings are particularly significant given the prevailing imbalance in modern Western diets, which are typically characterized by excessive intake of ω-6 PUFAs and a disproportionately high ω-6/ω-3 ratio. A balanced ω-6/ω-3 ratio is essential for maintaining physiological homeostasis and supporting normal development, whereas a persistent skew toward ω-6 fatty acids has been implicated in the etiology of numerous chronic disorders, including cardiovascular disease, cancer, and inflammatory and autoimmune conditions (Simopoulos, 2016a). By shifting the fatty acid profile toward a more favorable ω-3-dominant composition, the LTC method not only enhances the nutritional value of flaxseed oil but also contributes to the development of functional lipids that align with preventive health strategies. Moreover, this technique offers practical advantages: it eliminates the need for hazardous reagents, reduces environmental impact, and supports rapid, scalable processing through simple cooling and filtration steps, features that make it particularly suitable for integration into sustainable food and nutraceutical manufacturing pipelines (Soleimanian et al., 2015).

When juxtaposed, urea complexation excels in compositional precision but is constrained by low recovery and procedural complexity. LTC, on the other hand, delivers a pragmatic compromise, offering substantial PUFA enrichment, high yield, and favorable sustainability metrics. Thus, the choice of method should be guided by application-specific priorities: for pharmaceutical or clinical applications demanding ultra-pure omega-3 concentrates, urea complexation remains the gold standard. For food-grade oils, dietary supplements, and nutraceutical formulations where process safety, economic viability, and regulatory compliance are paramount, LTC presents a more balanced and scalable solution. Importantly, both approaches significantly improved the PUFA-to-SFA ratio compared to crude flaxseed oil, reaffirming their potential to enhance the health-promoting properties of plant-based lipid matrices. Their integration into lipid processing pipelines can be strategically tailored to support a spectrum of products across the nutrition–health–wellness continuum. Beyond their technical merits, these findings also contribute to a broader discourse on sustainable lipid engineering—demonstrating how classical physical and molecular separation techniques can be optimized to meet modern nutritional goals with precision and purpose.

4- Conclusion

This study demonstrated the efficacy of two crystallization-based techniques i.e., urea complexation and low-temperature crystallization (LTC), in enriching PUFAs in flaxseed oil. Urea complexation yielded the highest PUFA content (80%), with ALA rising to 63% and SFAs reduced to below 2%, though at the expense of low recovery (23%) and higher processing complexity. In contrast, LTC provided a slightly lower PUFA enrichment (70.45%) but achieved a much higher recovery (75%) and offered a safer, solvent-efficient, and scalable alternative. Overall, both methods significantly improved the PUFA:SFA ratio compared to crude oil, with urea complexation offering superior selectivity and LTC delivering greater process feasibility. The choice between them should be guided by end-use priorities: pharmaceutical precision versus food-industry practicality. These findings provide a foundation for developing customized lipid profiles to support functional food and nutraceutical innovations aligned with modern health and sustainability goals.

Author Contributions

All activities were performed by the authors.

Data availability

All data supporting the findings of this study are available within the article.

Conflict of Interest

The author confirms that there are no financial conflicts of interest or competing interests in this study.

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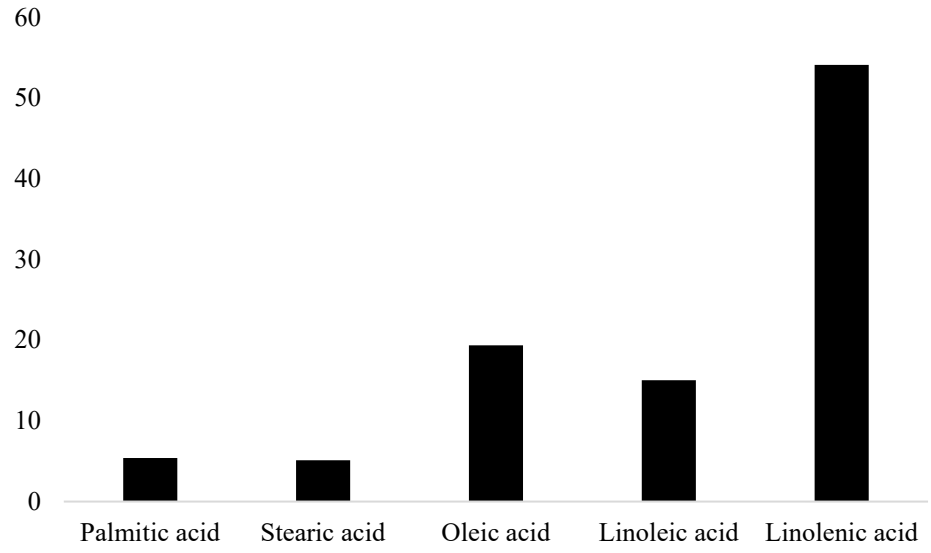

Fig. 1. Fatty acid composition of crude flaxseed (*Linum usitatissimum*) oil as determined by gas chromatography. Values represent the percentage of total identified fatty acid methyl esters (FAMEs).

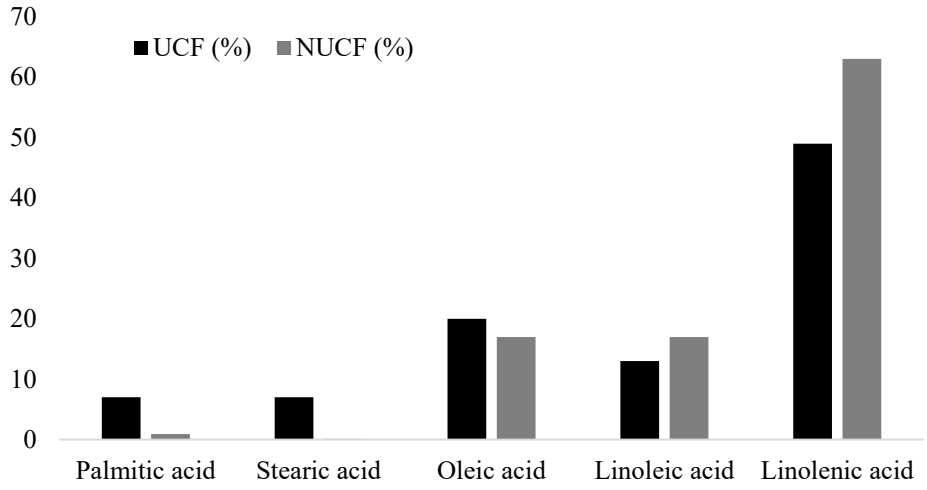

Fig. 2. Comparative fatty acid composition of urea-complexed (UCF) and non-urea-complexed (NUCF) fractions derived from flaxseed (*Linum usitatissimum*) oil following urea complexation. Data are expressed as percentage of total identified FAMEs.

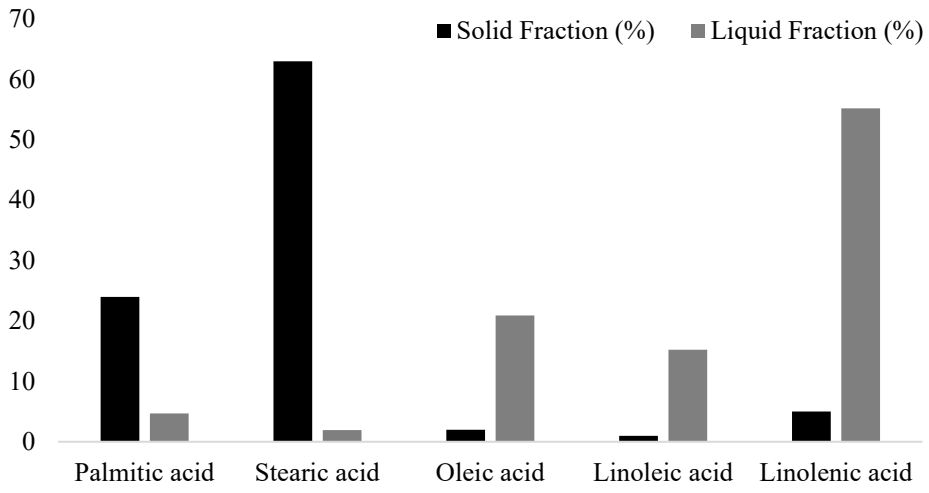

Fig. 3. Fatty acid composition of solid and liquid fractions obtained from flaxseed (*Linum usitatissimum*) oil following low-temperature crystallization. Percentages reflect the composition of total identified FAMEs.

Table 1. Fatty acid composition of flaxseed (*Linum usitatissimum*) oil fractions obtained by urea complexation and low-temperature crystallization

Parameter	**Crude oil**	**Urea complexation**		**Crystallization**	
	-	UCF	NUCF	SF	LF
SFAs	10.5%	14.0%	1.9%	87.0%	6.62%
UFAs	88.51%	82.0%	97.0%	8%	91.35%
ω-3/ω-6	3.6	3.76	3.70	5.0	3.62
SFA/UFA	0.12	0.17	0.02	10.88	0.072
SFA/PUFA	0.15	0.23	0.024	14.5	0.094
PUFA/MUFA	3.57	3.1	4.7	3.0	3.37
Recovery yield (%)	-	14.4%	23%	21%	75%

UCF: urea complexation fraction, NUCF: non-urea complexation fraction, SFAs: saturated fatty acids, SF: solid fraction, LF: liquid fraction, PUFAs: polyunsaturated fatty acids, MUFAs: monounsaturated fatty acids, UFAs: unsaturated fatty acids, ω-3/ω-6: ratio of omega-3 to omega-6 fatty acids, SFA/UFA: ratio of saturated fatty acids to unsaturated fatty acids, SFA/PUFA: ratio of saturated fatty acids to polyunsaturated fatty acids, PUFA/MUFA: ratio of polyunsaturated fatty acids to monounsaturated fatty acids.

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مقاله علمی_پژوهشی

غنی‌سازی اسیدهای چرب ضروری امگا-۳ از روغن کتان روغنی با استفاده از کمپلکس‌سازی اوره و تبلور سرد

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چکیده

روغن کتان (*Linum usitatissimum*) به عنوان یک منبع گیاهی سرشار، حاوی اسیدهای چرب ضروری است که به طور اخص شامل اسید لینولنیک (اُمگا-۳) و اسید لینولئیک (اُمگا-٦) می‌شود. هر دوی این اسیدهای چرب با طیف گسترده‌ای از منافع بهداشتی و سلامتی مرتبط هستند. در مطالعه حاضر، دو شیوه اصلی برای غنی‌سازی اسیدهای چرب چند غیراشباع، شامل تشکیل کمپلکس اوره و تبلور در دمای پایین، به منظور سنجش کارایی آن‌ها در افزایش میزان اسیدهای چرب ضروری روغن بذر کتان، به صورت مقایسه‌ای مورد ارزیابی قرار گرفتند. روش‌شناسی و تجزیهٔ ترکیبات نیمرخ اسیدهای چرب در نمونه‌های خام و بخش‌های فرآوری‌شده، با استفاده از روش کروماتوگرافی گازی–آشکارساز یونش شعله‌ای تعیین شدند. همچنین، بازده‌های بازیابی جهت ارزیابی کلی بازده فرآیند محاسبه گردیدند. تحلیل روغن خام نشان داد که این نمونه متشکل از ٥٤/۱۳ درصد آلفا-لینولنیک اسید، ۱٥/۰۲ درصد لینولئیک اسید، ۱۹/۳٦ درصد اولئیک اسید و ۱۰/٥ درصد اسیدهای چرب اشباع است. بنابراین، سهم کلی اسیدهای چرب ضروری در نیمرخ مزبور بالغ بر ٦۹/۱٥ درصد بوده است. یافته‌های مقایسه‌ای غنی‌سازی فرآیند تشکیل کمپلکس اوره به تولید بخش غیرکمپلکس‌شده با اوره منتهی گردید که ترکیب آن از نظر آلفا-لینولنیک اسید به ٦۳/۰ درصد و از نظر لینولئیک اسید به ۱۷/۰ درصد غنی شده بود و در عین حال، مقدار اسیدهای چرب اشباع را به ۱/۹ درصد کاهش داد. با این وجود، بازده کلی بازیابی این روش نسبتاً پایین بود؛ به طوری که ۲۳/۰ درصد از جرم در بخش غیرکمپلکس‌شده با اوره و ۱٤/٤ درصد در بخش کمپلکس‌شده با اوره بازیابی شد که در مجموع، حفظ جرم به ۳۷/٤ درصد محدود گردید. در مقابل، روش تبلور در دمای پایین بخش مایعی را حاصل نمود که حاوی ٥٥/۲ درصد آلفا-لینولنیک اسید و ۱٥/۲٥ درصد لینولئیک اسید بود و درصد اسیدهای چرب اشباع در آن به ٦/٦۲ درصد تقلیل یافت. بازده بازیابی حاصل از این روش به مراتب چشمگیرتر بود؛ زیرا ۷٥ درصد از جرم در فاز مایع و ۲۱ درصد در فاز بلورین حفظ شد که این امر متناظر با بازده کلی بازیابی ۹٦ درصد است. این اثر، نخستین ارزیابی مقایسه‌ای صریح این دو روش را برای غنی‌سازی گزینشی اسیدهای چرب ضروری از روغن بذر کتان ارائه می‌دهد و بر وجود یک موازنه حیاتی میان خلوص ترکیبی و بازیابی ماده تأکید می‌ورزد. نتایج حاصل از این پژوهش، راهنمای مستند و مبتنی بر شواهد را به منظور انتخاب راهبردهای غنی‌سازی که متناسب با اهداف خاص صنعتی یا تغذیه‌دارویی باشند، فراهم می‌کند.