



Optimization of Chia Seed Oil Extraction Using Microwave and Moisture Pretreatments: Impact on Oil Quality, Omega-3 Content, and Stability

Hasan Ahmad¹, Sodeif Azadmard-Damirchi^{1*}, Kazem Alirezalu¹

Abstract

Objectives: Chia seed oil is a nutritionally valuable functional oil rich in polyunsaturated fatty acids, particularly omega-3, whose health benefits strongly depend on oxidative stability during processing and storage. This study aimed to optimize chia seed oil extraction using microwave and moisture pretreatments through response surface methodology (RSM), with emphasis on maximizing oil yield, improving lipid quality, and enhancing omega-3 stability during processing and storage, as assessed by peroxide value (PV) and oxidative stability index (OSI).

Materials and Methods: An I-optimal response surface design was applied to investigate the effects of seed moisture content and microwave pretreatment time on oil content (%), PV, and OSI. Model adequacy was evaluated using analysis of variance and diagnostic statistical criteria. Optimal extraction conditions were identified through numerical optimization and experimentally validated. In addition, a 90-day storage stability study was conducted to compare oils produced under optimized and conventional conditions, monitoring PV and OSI changes during storage.

Results: The developed RSM models were statistically significant and showed strong predictive performance. Optimal conditions (approximately 8.05% seed moisture and 135.9 s microwave treatment) resulted in the highest oil yield while maintaining low PV and enhancing OSI. During storage, oil obtained under optimized conditions exhibited slower oxidative deterioration and greater resistance to lipid oxidation compared with conventionally extracted oil, indicating improved preservation of omega-3-rich polyunsaturated fatty acids, which are highly susceptible to oxidative degradation.

Conclusions: Microwave–moisture pretreatment optimization represents an effective strategy for producing high-quality chia seed oil with high content of omega-3 fatty acid, and improved oxidative stability during processing and storage, supporting its nutritional and health-related value for functional food and nutraceutical applications.

Keywords: Chia seed oil, Microwave pretreatment, Oxidative stability, Omega-3 content and stability, Functional food

Introduction

Chia (*Salvia hispanica* L.) is an herbaceous plant primarily cultivated as a semi-annual crop, with seeds noted for their high oil content, typically 25% to 35% (1,2). Chia seed oil is particularly rich in polyunsaturated fatty acids (PUFAs), with α -linolenic acid (C18:3n-3, omega-3) as the predominant component, comprising over 60% and, in some cases, up to approximately 68% of total fatty acids, which makes it valuable from nutritional and medicinal points of view (2). Other major fatty acids include linoleic acid, oleic acid, palmitic acid, and stearic acid, while saturated fatty acids account for only about 10%, resulting in a lipid profile highly favorable for nutritional and functional applications (3).

In addition to its fatty acid composition, chia seeds contain significant amounts of dietary fiber, high-quality proteins, and bioactive compounds such as phenolic acids and flavonoids, which have been associated with cardiovascular protection, anti-inflammatory effects, and improvements in lipid metabolism (4). These nutritional

and bioactive properties position chia seed oil as a valuable functional oil with potential health-promoting effects (5).

Despite these beneficial attributes, chia seed oil is highly susceptible to oxidative degradation due to its elevated PUFA content. Lipid oxidation leads to the loss of nutritionally important omega-3 fatty acids, deterioration of sensory quality, and formation of potentially harmful oxidation products, ultimately limiting shelf life and functional value of the oil (6,7).

Conventional extraction methods, including cold pressing and solvent extraction, are widely used for chia seed oil recovery, but present notable limitations (3). Solvent extraction is often associated with long processing times, high energy consumption, safety and environmental concerns, and the risk of solvent residues in the final product (8). While hot pressing may enhance oil extraction yield, elevated temperatures can accelerate oxidative reactions and degrade heat-sensitive bioactive compounds, whereas cold pressing better preserves nutritional quality but typically results in lower extraction



efficiency (9).

These challenges have driven the development of innovative extraction technologies to improve oil yield while preserving nutritional quality and oxidative stability (10). At the same time, pretreatment methods, especially moisture conditioning, are known to boost mass transfer, break down cell walls, and increase the recovery and stability of lipids and bioactive compounds (11). New techniques such as supercritical fluid extraction, ultrasound-assisted extraction, microwave-assisted extraction (MAE), and electric-field treatments have demonstrated significant potential for addressing the limitations of traditional methods (12-14).

Among these approaches, MAE has attracted increasing attention due to its rapid heating, reduced processing time, and improved extraction efficiency (11,15). MAE operates via dielectric heating, in which microwave energy interacts with polar molecules within the seed matrix, resulting in rapid volumetric heating, increased internal pressure, and cell wall rupture, thereby facilitating oil release (16). The presence of moisture within the seed matrix plays a critical role in this process by enhancing microwave energy absorption and influencing both extraction efficiency and oxidative behavior of the extracted oil (11,16).

Response surface methodology (RSM) is a powerful statistical tool widely used to optimize complex food processing systems, enabling the simultaneous evaluation of multiple variables and their interactions with a limited number of experimental runs (17). In edible oil processing, RSM enables the identification of optimal conditions that maximize oil yield while maintaining critical quality indicators such as peroxide value (PV) and oxidative stability index (OSI), which are directly related to omega-3 stability and shelf-life quality (10,11).

Despite growing interest in microwave pretreatment and moisture conditioning, comprehensive studies integrating these approaches with RSM optimization of chia seed oil—particularly with emphasis on oxidative quality, omega-3 stability, and nutritional value—remain limited (11,15). Therefore, the present study aims to optimize chia seed oil extraction using microwave and moisture pretreatments through RSM, with the objective of maximizing oil yield while improving oxidative stability and lipid quality, as evaluated by PV and OSI.

Although microwave pretreatment has been investigated to improve oil extraction yield and quality in PUFA-rich oilseeds, comprehensive studies integrating microwave and moisture conditioning within an advanced experimental design framework and extending the evaluation to storage-based nutritional stability assessment for chia seed oil remain limited (11,15). In this study, an I-optimal experimental design was employed to minimize prediction variance and enable multi-response optimization of oil extraction yield, PV, and OSI (17). The optimized conditions were further validated through extended storage experiments coupled with direct fatty

acid stability analysis. This integrated framework provides a more systematic evaluation of extraction efficiency and nutritional stability than previously reported approaches.

Materials and Methods

This section outlines the materials used and the methodological framework adopted in the study. The procedures include sourcing and preparing chia seeds, designing the experimental protocol incorporating microwave and moisture pretreatments, the cold-press oil extraction protocol, and analytical methods for determining oil yield, PV, and OSI. Each step is detailed to ensure reproducibility and clarity regarding the optimization and evaluation of chia seed oil extraction and quality parameters.

Materials

Chia seeds (*Salvia hispanica* L.) were purchased from a local market in Tabriz, Iran, and stored in a cool, dry place until use. Prior to extraction, seeds were manually cleaned to remove dust and foreign materials, and no chemical treatment was applied. The initial moisture content of the seeds was 5.3% (dry basis, % w/w), determined by the AOAC loss-on-drying method using an air oven at 103–104 °C until constant weight (18). All reagents and solvents used in the experimental procedures were of analytical grade and were obtained from Merck (Darmstadt, Germany).

Design of Experiment

To systematically investigate and optimize the extraction process, RSM was employed as a robust statistical approach. Two key variables were considered as independent factors: seed moisture content (MOS, 0–10%) and microwave treatment time (TIME, 0–180 s). The experiment targeted three main responses: oil content (%), PV as an indicator of primary oxidation, and OSI as a measure of resistance to lipid oxidation.

An I-optimal response surface design based on the coordinate-exchange algorithm was applied to efficiently explore the experimental domain and minimize prediction error. The experimental matrix consisted of 16 runs, including replicated center points to ensure reliable estimation of pure error and facilitate lack-of-fit testing (see Table 1 for details on factor ranges and design structure).

For each response, a second-order (quadratic) polynomial regression model was fitted to the data, generally expressed as:

$$Y = \beta_0 + \sum_j \beta_j X_j + \sum_j \beta_{jj} X_j^2 + \sum_{(i \neq j)} \beta_{ij} X_i X_j$$

where Y is the response (oil content, PV, or OSI), X_i and X_j represent the coded independent variables (MOS and TIME), and β_0 , β_j , β_{jj} , and β_{ij} are the coefficients for the constant, linear, quadratic, and interaction effects,

Table 1. I-Optimal Response Surface Design Matrix With the Experimental And Predicted Values for Chia Seed Oil Responses

Run order	Independent variables		Oil content (%)		Peroxide value (meq O ₂ /kg)		OSI (h)	
	MOS (%)	TIME (s)	Experimental	Predicted	Experimental	Predicted	Experimental	Predicted
1	0	180	32.20	32.32	4.74	4.74	12.00	12.11
2	5	55.99	28.50	28.65	3.01	3.01	11.10	10.99
3	10	90	32.10	32.11	4.74	4.74	12.00	12.07
4	8.05	135.9	34.00	33.93	5.92	5.92	12.10	11.83
5	10	180	32.90	33.25	7.98	7.98	10.90	10.94
6	5	124.2	31.80	32.94	4.85	4.85	11.00	11.72
7	0	0	20.10	20.56	1.50	1.50	10.00	9.97
8	10	90	32.70	32.11	4.74	4.74	12.10	12.07
9	5	180	34.50	33.40	6.36	6.36	11.40	11.40
10	1.9	45	27.00	26.22	2.46	2.46	10.50	10.67
11	5	0	22.50	22.07	1.50	1.50	9.00	9.49
12	10	0	20.10	20.34	1.50	1.50	9.40	9.42
13	0	90	28.70	28.27	3.12	3.12	11.30	11.29
14	2.3	145.8	31.90	32.44	4.73	4.73	12.20	11.74
15	5	90	31.10	31.30	3.93	3.93	11.70	11.51
16	5	0	21.90	22.07	1.50	1.50	10.00	9.49

respectively.

Model adequacy and validity were evaluated using analysis of variance (ANOVA), coefficients of determination (R^2 , adjusted R^2 , predicted R^2), coefficient of variation (CV), adequate precision, and lack-of-fit tests. Regression equations were developed in both coded and actual terms to characterize the relationships between variables and responses. Diagnostic checks were performed to ensure the reliability and stability of the models.

Numerical optimization was performed using Derringer's desirability function (17) to identify optimal processing conditions that maximize oil content and OSI while minimizing PV. To verify the adequacy and predictive accuracy of the developed models, experimental validation was conducted by performing duplicate experiments under the predicted optimal conditions, and the experimental results were subsequently compared with the corresponding model-predicted values.

All aspects of experimental design and statistical analysis were carried out using Design-Expert software, version 10.0.7 (Stat-Ease Inc., Minneapolis, USA).

Moisture Pretreatment Procedure

The initial moisture content of chia seeds was adjusted to the required levels according to the experimental design prior to microwave treatment. Predetermined amounts of distilled water were uniformly applied to the seed samples to achieve the desired moisture content. The moistened seeds were thoroughly mixed, sealed in polyethylene bags, and stored at 4 °C for 24 hours to ensure uniform moisture distribution throughout the seed matrix before

microwave processing (19). After the equilibration period, samples were removed from cold storage and allowed to reach ambient temperature prior to MAE. This moisture pretreatment was applied to enhance microwave energy absorption and promote more efficient oil release during extraction.

Microwave Pretreatment

Chia seeds were first adjusted to the target moisture levels as described in the experimental design. For each experimental run, 100 g of the moisture-conditioned seeds was transferred to a heat-resistant glass container, evenly spread to ensure consistent microwave exposure, and subjected to microwave pretreatment for the specified exposure time (0–180 s). Microwave pretreatment was conducted using a domestic microwave oven operating at 2.45 GHz and a constant power output of 800 W. During microwave treatment, seed temperature was not directly monitored quantitatively; however, processing conditions were carefully controlled to prevent visible scorching or overheating, and all handling conditions were kept constant across runs. Immediately after pretreatment, the seeds were allowed to cool naturally to room temperature before undergoing the subsequent solvent-free cold-press extraction. This pretreatment was applied to alter the seed microstructure and enhance oil release during pressing, as reported for microwave-treated oilseeds (20).

Cold-Press Oil Extraction

Chia seeds were subjected to solvent-free cold-press extraction immediately after microwave pretreatment using a laboratory-scale hydraulic oil press (Model 6YZ-

180). The press was operated with a rated motor power of 1.1 kW and a batch capacity of 3.5 kg. The laboratory-scale hydraulic oil press used for solvent-free cold-press extraction is shown in Figure 1.

For each experimental run, the press was operated for approximately 6 minutes per batch under constant mechanical conditions. The pressing pressure was fixed according to the manufacturer's default operating settings to ensure consistency across all experiments.

The temperature of the expressed oil was continuously monitored using an infrared thermometer. It was maintained below 45 °C throughout the extraction process to minimize thermal degradation of heat-sensitive compounds and oxidative reactions. No external heating was applied during pressing.

The extracted oil was collected in amber glass containers and allowed to stand at room temperature for 24 hours to facilitate sedimentation of suspended solids, followed by filtration before further analysis.

Determination of Oil Yield

Oil yield was determined gravimetrically. Following cold-press extraction and clarification, the recovered oil mass was precisely measured using an analytical balance. For each experimental run, oil yield was calculated as the percentage of the extracted oil mass relative to the initial dry mass of chia seeds, using the equation:

$$\text{Oil yield (\%)} = (\text{Mass of extracted oil} / \text{Initial dry mass of chia seeds}) \times 100$$

All measurements were conducted in triplicate, and the mean values were used for statistical analysis.

Determination of Peroxide Value

The PV of the extracted chia seed oil was determined using the standard iodometric titration procedure described in the AOCS method (21). In brief, a known quantity of oil was dissolved in a mixture of acetic acid and chloroform, to which a saturated potassium iodide solution was added. The liberated iodine was then titrated with standardized sodium thiosulfate solution, using starch as an indicator.

PV was calculated and expressed as milliequivalents of active oxygen per kilogram of oil (meq O₂/kg oil). All measurements were performed in triplicate, and mean values were used for statistical analysis.

Determination of Oxidative Stability Index

The OSI of chia seed oil samples was determined using a Rancimat apparatus in accordance with the AOCS Official Method Cd 12b-92 (22). Oil samples (3.0 g) were subjected to accelerated oxidation at 110 °C with an air flow rate of 20 L/h. The induction time, expressed in hours, was automatically recorded by the instrument and used as an indicator of oxidative stability. All measurements were conducted in triplicate, and mean values were used for statistical analysis.



Figure 1. Laboratory-Scale Cold-Press Extraction System Used for Solvent-Free Recovery Of Chia Seed Oil.

Conventional (Control) Oil Extraction

For comparison, a conventional control oil was prepared using solvent-free cold-press extraction, without microwave or moisture pretreatments. In this process, chia seeds were pressed directly at their native moisture content (5.3%, dry basis), without any prior conditioning or microwave exposure. The cold-press extraction conditions—including press type, operation time, and temperature control below 45 °C—were identical to those used for the optimized samples. This control treatment represents the traditional cold-press extraction method and served as a baseline for evaluating the effects of microwave–moisture pretreatment on oil quality and storage stability.

Storage Stability Study

Following extraction under both optimized and control conditions, chia seed oil samples were immediately transferred into sealed amber glass bottles and stored in the dark at 25 ± 2 °C. The oxidative and physicochemical stability of the oils was evaluated over a 90-day storage period. Samples were collected at predetermined intervals (0, 30, 60, and 90 days) for analysis.

PV was determined by iodometric titration according to the AOCS Official Method Cd 8b-90 (21) and expressed as milliequivalents of O₂ per kilogram of oil (meq O₂/kg). Free fatty acid (FFA) content was measured following the AOCS Official Method Ca 5a-40 (23) and expressed as a % oleic acid. Total phenolic content (TPC) was quantified using the Folin–Ciocalteu method with gallic acid as the standard and expressed as mg gallic acid equivalents per kg of oil (mg GAE/kg) (24). Chlorophyll and carotenoid contents were determined spectrophotometrically according to the method described by Mínguez-Mosquera et al (25).

Fatty Acid Composition Analysis

The fatty acid composition of chia seed oil obtained under optimized and control extraction conditions was

determined by gas chromatography (GC) after conversion of oil samples to fatty acid methyl esters (FAMES) according to ISO 12966-2:2017 (26,27). Identification of FAMES was performed using a gas chromatograph equipped with a flame ionization detector (FID) and a split/splitless injector, fitted with a fused-silica capillary column BPX70 (50 m × 0.22 mm i.d., 0.25 µm film thickness; SGE, Austin, TX, USA). The injector and detector temperatures were set at 230 °C and 250 °C, respectively. Helium was used as the carrier gas and nitrogen as the make-up gas, with a total gas flow rate of 30 mL/min. The oven temperature program started at 158 °C and was increased to 220 °C at a rate of 2 °C/min, then held for 5 min, as previously reported (28). Individual FAMES were identified by comparing retention times with those of authentic reference standards, and peak areas were expressed as relative percentages of total identified fatty acids. The analysis focused on major saturated and unsaturated fatty acids, with particular emphasis on α -linolenic acid (C18:3n-3, omega-3), due to its high nutritional value and susceptibility to oxidative degradation (3).

Statistical Analysis of Storage Data

The effect of storage time on oxidative stability and quality parameters (including PV, OSI, FFA, TPC, chlorophyll, and carotenoids) was evaluated using one-way ANOVA, followed by Tukey's post hoc test to determine significant differences between storage intervals. All measurements were performed in triplicate. Differences were considered statistically significant at $P < 0.05$.

Results and Discussion

Validation of the Optimized Extraction Conditions

The adequacy and predictive accuracy of the developed RSM models were verified by experimental validation conducted under the predicted optimal extraction conditions (MOS = 8.05% and TIME = 135.9 s). The experimentally obtained values for oil content, PV, and OSI under the optimized extraction conditions closely matched the model-predicted values, with relative errors below 3%, confirming the reliability and predictive accuracy of the developed models (Table 2).

The results demonstrated a close agreement between experimental and predicted values for all responses, with relative errors below 3%. This high level of agreement confirms the reliability, robustness, and predictive capability of the developed response surface models for optimizing chia seed oil extraction.

Effect of Microwave Pretreatment and Moisture Content on Oil Yield

The oil yield of chia seeds was markedly influenced by both seed moisture content (MOS) and microwave pretreatment time (TIME). The experimental results revealed considerable variation in oil recovery across the investigated domain, demonstrating that properly adjusted pretreatment conditions play a critical role in enhancing oil liberation during subsequent cold pressing.

In general, higher oil yields were obtained at moderate moisture levels combined with appropriate microwave exposure times. This behavior can be explained by the fact that water molecules act as efficient microwave absorbers, resulting in rapid volumetric heating within the seed matrix. The resulting internal pressure disrupts the microstructure and ruptures cell walls, significantly facilitating oil migration and release during mechanical pressing. Recent studies have confirmed that controlled microwave pretreatment improves mass transfer and extraction efficiency in oil-bearing seeds by modifying cellular structures and weakening oil–matrix interactions (5,7).

The experimental and model-predicted oil content values and residuals are summarized in Table 3.

Analysis of response trends indicated the presence of an optimal operating region. While increasing microwave exposure initially enhanced oil recovery, excessive treatment time—particularly at elevated moisture levels—did not provide further improvement and, in some cases, slightly reduced the yield. This behavior has been attributed to structural collapse of seed tissues and partial thermal degradation of oil-bearing components under excessive thermal stress (5,7).

As illustrated in Figures 2 and 3, oil yield increased progressively with microwave pretreatment time at moderate moisture levels, reaching a maximum within the central region of the experimental domain. The response surface demonstrates a clear synergistic effect between moisture content and microwave exposure, confirming that neither factor alone is sufficient to achieve maximum oil recovery. The contour plot further identifies a distinct optimal zone where oil yield is maximized, beyond which increases in treatment intensity yield diminishing returns, likely due to partial collapse of oil-bearing structures and localized thermal degradation.

Overall, these findings confirm that balanced control of moisture conditioning and microwave pretreatment is essential for maximizing chia seed oil yield while

Table 2. Experimental Validation of the Optimized Extraction Conditions by Comparing Predicted and Experimental Values

Response	Predicted value	Experimental value	Relative error (%)
Oil content (%)	33.93	34.00	0.21
PV (meq O ₂ /kg)	5.92	5.92	0.00
OSI (h)	11.83	12.10	2.28

Note: Values represent the mean of duplicate validation experiments (n = 2).

Table 3. I-Optimal Design Matrix With Experimental and Predicted Oil Content Values for Chia Seed Oil

Run	MOS (%)	TIME (s)	Oil content (Exp., %)	Oil content (Pred., %)	Residual
1	0.00	180	32.20	32.32	-0.12
2	5.00	55.9903	28.50	28.65	-0.15
3	10.00	90	32.10	32.11	-0.01
4	8.05	135.9	34.00	33.93	0.07
5	10.00	180	32.90	33.25	-0.35
6	5.00	124.2	31.80	32.94	-1.14
7	0.00	0	20.10	20.56	-0.46
8	10.00	90	32.70	32.11	0.59
9	5.00	180	34.50	33.40	1.10
10	1.90	45	27.00	26.22	0.78
11	5.00	0	22.50	22.07	0.43
12	10.00	0	20.10	20.34	-0.24
13	0.00	90	28.70	28.27	0.43
14	2.30	145.8	31.90	32.44	-0.54
15	5.00	90	31.10	31.30	-0.20
16	5.00	0	21.90	22.07	-0.17

maintaining process stability. The identification of this optimal operating region provides a robust basis for subsequent multi-response optimization and detailed evaluation of oxidative quality parameters.

Effect of Microwave Pretreatment and Moisture Content on Oxidative Quality of Chia Seed Oil

Effect on Peroxide Value

PV, as a primary indicator of lipid oxidation, was significantly affected by both moisture content (MOS) and microwave pretreatment time (TIME). The statistical analysis demonstrated that microwave exposure time had the strongest influence on PV ($P < 0.0001$), while the interaction between MOS and TIME also contributed significantly to the response behavior.

As presented in Table 3, PV values increased progressively with increasing microwave pretreatment time, particularly at higher moisture levels. This behavior is also illustrated in Figures 4 and 5, where PV rises toward longer exposure

times and higher MOS. This trend is attributed to enhanced molecular mobility and localized heating within the seed matrix, which accelerates oxidative reactions in PUFAs when excessive thermal energy is applied.

The fitted interaction model adequately described the experimental data, yielding a high coefficient of determination and confirming its predictive reliability. These results emphasize the importance of limiting microwave exposure to avoid excessive oxidation while still benefiting from improved oil release.

These results indicate the need to balance oil recovery and oxidative stability, as conditions that maximize oil yield do not always correspond to minimal oxidation.

Effect on Oxidative Stability Index

The OSI, indicating how well oil resists oxidative degradation, was heavily affected by the pretreatment conditions. OSI improved with moderate microwave exposure, peaking in the central part of the experimental

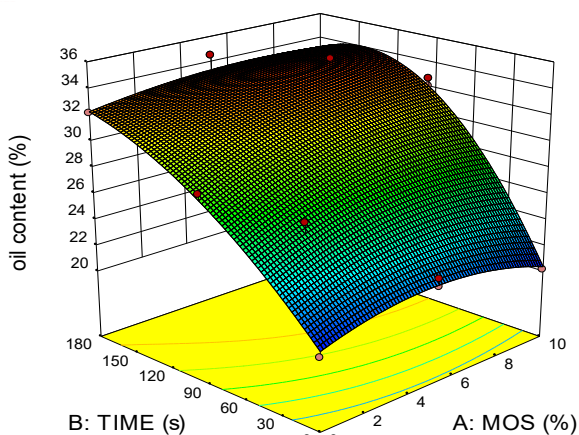


Figure 2. 3D Response Surface Plot Showing the Effect of MOS and TIME on Oil Yield.

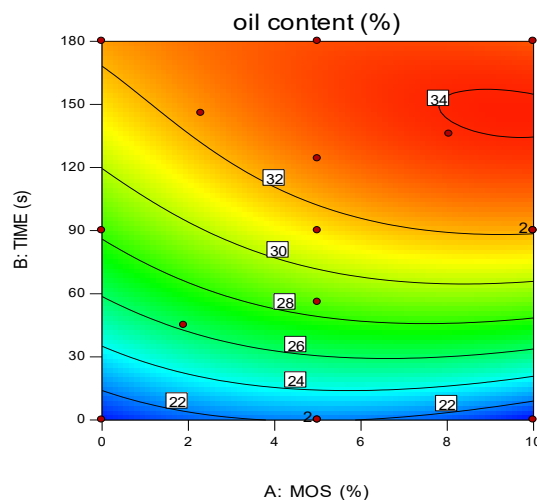


Figure 3. Contour Plot Illustrating the Optimal Region for Maximum Oil Yield.

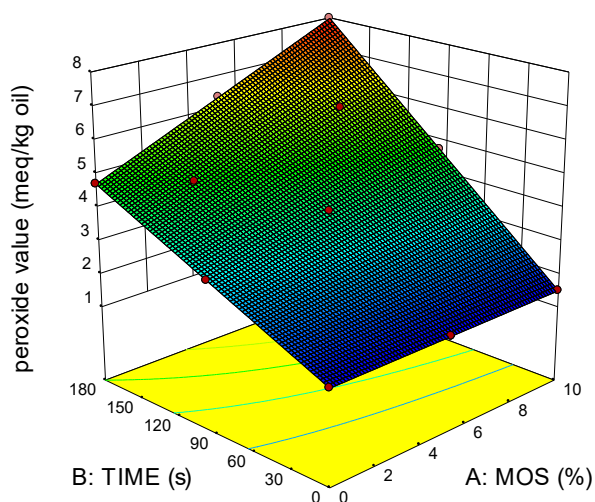


Figure 4. 3D Response Surface Plot Showing the Effect of MOS and TIME on Peroxide Value.

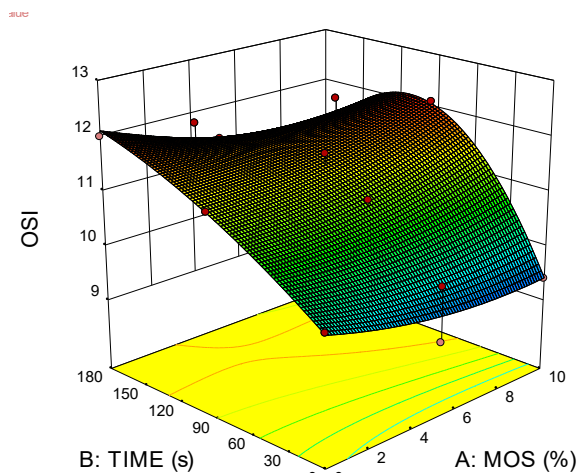


Figure 6. 3D Response Surface Plot Showing the Effect of MOS and TIME on OSI.

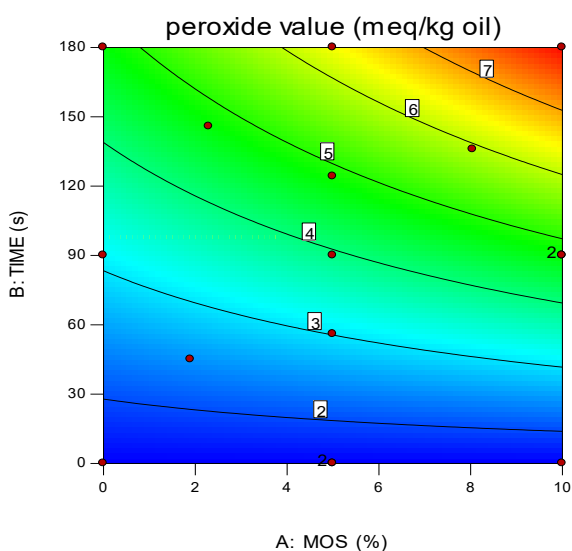


Figure 5. Contour Plot Illustrating Optimal Low-Peroxide Value Region.

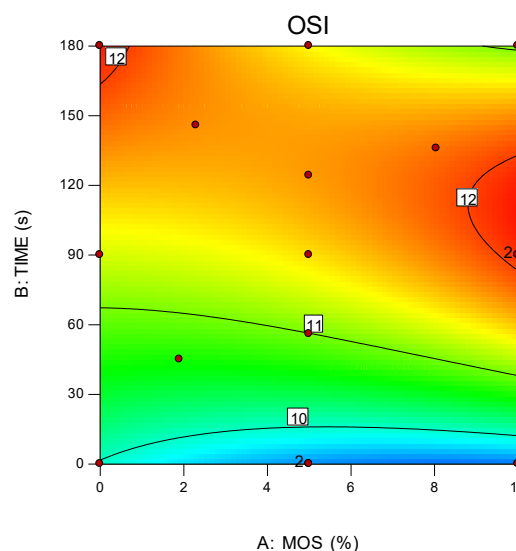


Figure 7. Contour Plot Identifying The Region Of Maximum OSI.

range, but decreased with longer treatment durations. As shown in Figures 6 and 7, the highest OSI values were observed at intermediate levels of MOS and TIME, suggesting an optimum stability region.

This behavior suggests that controlled microwave pretreatment promotes the release of endogenous antioxidants and enhances oil stability; however, excessive exposure results in thermal degradation of protective compounds, thereby reducing oxidative resistance.

The opposing trends observed for PV and OSI further underscore the need to identify an optimal pretreatment window that simultaneously maximizes oil yield and oxidative stability while minimizing oxidation.

Comparative Storage Stability of Optimized Versus Control Oils

The storage stability results (Table 4) revealed significant

and nutritionally relevant differences between chia seed oils extracted under optimized and control conditions throughout the 90-day storage period. The control oil was obtained by solvent-free cold-press extraction without microwave or moisture pretreatments, as described in *Conventional (Control) Oil Extraction* Section. These findings highlight how microwave–moisture pretreatment can help protect the nutritional quality and oxidative stability of omega-3-rich lipids and bioactive compounds in chia seed oil during storage, ensuring you can continue to enjoy its health benefits.

Impact of Optimized Extraction Conditions on Peroxide Value During Storage

PV, a primary indicator of lipid oxidation and an indirect marker of omega-3 degradation, exhibited distinct trends between the optimized and control oils during storage.

Table 4. Comparative Storage Stability of Optimized Versus Control Oils

Day	Sample	PV (meq O ₂ /kg)	FFA (%)	Total phenolic (mg/kg)	Chlorophyll (mg/kg)	Carotenoids (mg/kg)
0	Optimized	5.9aD*	0.6a	120aA	4.5bA	1.2bA
	Control	1.5bD	0.4b	95bA	8.0aA	3.8aA
30	Optimized	8.0aC	0.6a	100aB	4.1bB	1.1bA
	Control	6.0bC	0.4b	75bB	6.1aB	3.0aB
60	Optimized	10.5bB	0.7a	95aC	3.5bC	1.0bA
	Control	11.0aB	0.6b	68bC	5.0aC	2.3aC
90	Optimized	13.0bA	0.7a	75aD	3.3bC	0.7bB
	Control	16.0aA	0.8a	55bD	4.4aD	1.6aD

*Different lowercase letters show differences between oil samples, and different capital letters show differences between oil samples during storage ($P < 0.05$).

On day 0, the optimized oil displayed a higher initial PV (5.9 meq O₂/kg) than the control oil (1.5 meq O₂/kg). This difference is likely attributable to mild oxidative initiation resulting from microwave-induced cellular disruption and increased exposure of PUFAs.

Despite this initial rise, the optimized oil showed notably better long-term oxidative stability. Throughout the 90-day storage period, peroxide formation progressed more slowly, with PV reaching 13.0 meq O₂/kg after 90 days, versus 16.0 meq O₂/kg in the control oil. This indicates that, despite causing some early oxidation, the optimized extraction conditions ultimately improved the oil's resistance to lipid peroxidation over time of storage.

The slower rise in PV is particularly important nutritionally because it suggests better preservation of omega-3 fatty acids, which are highly susceptible to oxidation. The improved oxidative stability of the optimized oil likely results from higher retention of natural antioxidants and a more regulated oxidative process. Similar protective effects of optimized microwave pretreatment on oxidative stability and lipid quality have been documented by Liu et al (15).

Statistical analysis using one-way ANOVA showed that the rate of PV increase during storage was significantly lower ($P < 0.05$) in the optimized oil than in the control oil.

Impact of Optimized Extraction Conditions on Free Fatty Acid During Storage

During the 90-day storage period, the FFA levels remained relatively stable in both the optimized and control oils, with only slight increases observed (see Table 4). By the end of storage, the optimized oil showed FFA levels that were similar to or slightly lower than the control oil (0.7% compared to 0.8% at day 90). This indicates that microwave-moisture pretreatment did not cause significant hydrolytic breakdown of triacylglycerols.

This stability is important for nutrition because excessive FFA increases can compromise oil quality, cause off-flavors, and lower consumer acceptance. As previous studies have shown, controlled microwave pretreatment under optimal conditions does not increase FFA levels (9,19). The consistent FFA levels in the optimized oil

suggest that the lipid structure is well-preserved with minimal enzymatic or chemical breakdown, which helps maintain the oil's shelf life and nutritional value, especially in omega-3-rich chia seed oil.

Impact of Optimized Extraction Conditions on Total Phenolic Content During Storage

TPC decreased gradually in both oils during storage; nevertheless, the optimized oil consistently maintained significantly higher phenolic levels than the control oil at all sampling points (Table 4). On day 0, the phenolic content was 120 mg/kg in the optimized oil versus 95 mg/kg in the control. After 90 days, the respective values were 75 mg/kg and 55 mg/kg.

Phenolic compounds are vital nutrients and physiological agents that act as natural antioxidants, preventing lipid oxidation and protecting omega-3 fatty acids from breakdown. The greater retention of phenolics in the optimized oil results from improved extraction methods and controlled thermal exposure during microwave pretreatment. Similar links between extraction methods, phenolic content, and antioxidant activity have been observed in cold-pressed plant oils (5,15). The increased phenolic levels in the optimized oil likely played a major role in its enhanced oxidative stability and nutritional benefits during storage (7).

Impact of Optimized Extraction Conditions on Chlorophyll and Carotenoid Contents During Storage

Pigment analysis further confirmed the trends we saw in how the chia seed oil stayed fresh and nutritious during storage. Throughout the storage period, the optimized oil consistently had lower levels of chlorophyll and carotenoids compared to the control oil. For instance, on day 0, the chlorophyll content in the optimized oil was 4.5 mg/kg, while the control oil measured 8.0 mg/kg. Likewise, carotenoid levels were 1.2 mg/kg in the optimized oil versus 3.8 mg/kg in the control.

Lowering chlorophyll levels can be beneficial for keeping oils fresh longer, because chlorophyll and its related compounds can act as pro-oxidants. They can speed up photo-oxidation and cause lipid peroxidation, especially in oils rich in PUFAs like omega-3s. The decrease in

chlorophyll seen in the optimized oil suggests that the microwave–moisture pretreatment effectively removes or breaks down these pro-oxidative pigments, helping to enhance the oil's stability during storage.

At the same time, having moderate levels of carotenoids—which are known for their antioxidant and health benefits—shows a good balance between reducing harmful pro-oxidative pigments and keeping valuable antioxidants intact. Similar findings have been seen in cold-pressed vegetable oils, where controlled pigment levels lead to better stability and quality. Overall, these results suggest that the optimized microwave–moisture pretreatment helps improve pigment balance, which in turn supports the stability and nutritional value of chia seed oil as it's stored.

Link Between Phenolic Content, Oxidative Stability, and Peroxide Value

Correlation analysis confirmed that higher TPC correlates with lower PVs and a higher OSI during storage. The differences in oxidative stability between the optimized and control oils can be explained by variations in TPC and pigment composition. Phenolic compounds are known for their ability to scavenge radicals and chelate metals, which helps inhibit lipid oxidation's initiation and propagation phases (6,9). The greater retention of phenolics in the optimized oil aligned with its higher OSI values and a slower increase in PV over time (15,19). Conversely, the control oil's lower phenolic levels and higher pro-oxidant pigments like chlorophyll likely sped up peroxide formation during storage (25). These results highlight a synergistic relationship between phenolic antioxidants, reduced peroxide formation, and improved oxidative stability in chia seed oil.

Changes in Fatty Acid Composition during Storage

The fatty acid composition of chia seed oil extracted under optimized and control conditions was evaluated at the beginning of storage (Day 1) and after 90 days to assess the stability of nutritionally important fatty acids, with particular emphasis on α -linolenic acid (C18:3n-3, omega-3). The results are summarized in Table 5, and a representative GC chromatogram of FAMES is shown in Figure 8.

At Day 1, α -linolenic acid was the predominant fatty acid in both samples, accounting for 58.1% in the optimized oil and 60.4% in the control oil, which is consistent with the typical fatty acid profile reported for chia seed oil (3,4). After 90 days of storage, α -linolenic acid decreased in both oils, consistent with oxidative degradation of omega-3 PUFAs during storage (7).

However, when relative loss was considered, the optimized oil showed better retention of α -linolenic acid during storage (a decrease of 4.1 percentage points) compared with the control oil (a decrease of 5.3 percentage points). Although the absolute α -linolenic acid content

Table 5. Fatty Acid Composition (%) of Chia Seed Oil Under Optimized and Control Extraction Conditions at Day 1 and Day 90 of Storage

Fatty acid	Day 1		Day 90	
	Control	Optimum	Control	Optimum
C16:0	6.5bB*	7.2aB	8bA	9.8aA
C18:0	3.8bB	4.9aA	5.4aA	5.0bA
C18:1	8.3aB	8.5aB	10.6bA	13.1aA
C18:2	14.9aA	13.6bA	12.7aB	11.9bB
C18:3	60.4aA	58.1bA	55.1aB	54.0aB

*Different lowercase letters show differences between oil samples, and different capital letters show differences between oil samples during storage ($P < 0.05$).

after 90 days remained slightly higher in the control oil (55.1%) than in the optimized oil (54.0%), the lower relative loss in the optimized sample suggests improved omega-3 stability under the optimized pretreatment/extraction conditions. Similar trends have been reported for microwave-treated oilseeds, where improved oxidative stability was associated with reduced degradation of PUFAs during storage (15,20).

Changes in other fatty acids followed expected oxidative patterns. Saturated fatty acids (C16:0 and C18:0) showed slight increases, which are commonly attributed to preferential oxidation of unsaturated fatty acids rather than actual formation of saturated species (5). Oleic acid (C18:1) increased modestly, particularly in the optimized oil, reflecting its higher oxidative stability compared with PUFAs (6,7).

Overall, the fatty acid composition remained within the typical range reported for chia seed oil, indicating that the optimized microwave–moisture pretreatment before cold-press extraction did not adversely affect lipid composition and may contribute to improved omega-3 stability during storage.

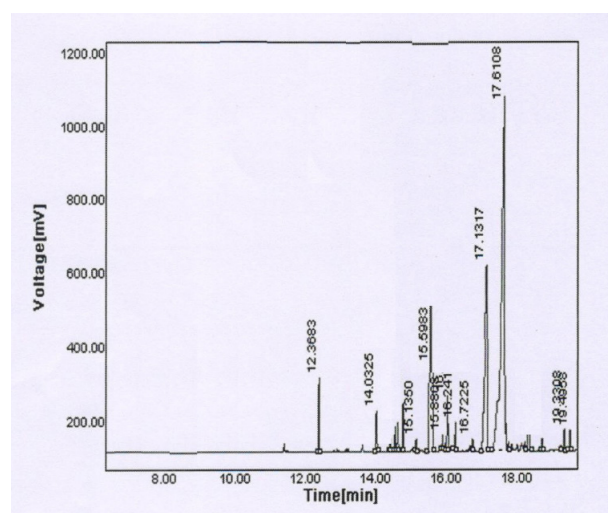


Figure 8. Representative Gas Chromatogram of Fatty Acid Methyl Esters of Chia Seed Oil Extracted Under Optimized Conditions (Day 1 of Storage).

Implications for Scale-up and Economic Feasibility

From a practical perspective, the proposed microwave-moisture pretreatment can be integrated into industrial cold-pressing lines as a short conditioning step before pressing. Compared with solvent-based extraction, this approach avoids solvent purchase, recovery operations, and concerns about solvent residues, thereby reducing operational complexity and improving process safety and environmental performance. The microwave step is time-efficient and can be implemented using continuous microwave systems (e.g., belt or screw-conveyor microwave units) following moisture equilibration in sealed containers, consistent with conditioning practices reported for oilseeds. Under the optimized conditions (8.05% moisture; 135.9 s; 800 W), the indicative electrical energy input is ~0.03 kWh per 50 g of seeds (~0.60 kWh/kg), suggesting a moderately energetic pretreatment while providing observed improvements in oxidative stability and omega-3 retention during storage. Overall, the combination of solvent-free pressing with a brief microwave-moisture conditioning step offers a scalable route to produce higher-stability chia oil for functional food applications (8,19,20).

Conclusions

This study successfully improved the process of extracting oil from chia seeds by using a combination of microwave pretreatment and moisture conditioning. The RSM helped identify key factors, such as seed moisture content and microwave treatment duration, that significantly affected both the amount of oil extracted and its oxidative stability. The best results were achieved with roughly 8% seed moisture and about 136 seconds of microwave treatment, leading to a high oil yield while keeping peroxide levels low and boosting the oil's stability.

Notably, oils produced with these optimized parameters showed better storage stability, demonstrated by slower oxidation rates, better preservation of antioxidant components, lower levels of pro-oxidative pigments, and stable FFA content over 90 days. These traits are especially important for omega-3-rich oils, as improved oxidative stability helps maintain their nutritional and health benefits.

In summary, the optimized microwave-moisture pretreatment is an effective method for producing high-quality chia seed oil with improved oxidative stability throughout processing and storage. This increased stability is demonstrated by lower PVs, a higher OSI, better retention of natural antioxidants, and less degradation of omega-3 PUFAs during storage. These results highlight the potential for applying this optimized oil in functional foods and nutraceutical products.

Authors' Contribution

Conceptualization: Hasan Ahmad, Sodeif Azadmard-Damirchi.

Data curation: Hasan Ahmad

Formal analysis: Hasan Ahmad, Kazem Alirezalu.

Investigation: Hasan Ahmad

Methodology: Hasan Ahmad

Resources: Sodeif Azadmard-Damirchi.

Software: Hasan Ahmad.

Supervision: Sodeif Azadmard-Damirchi.

Validation: Hasan Ahmad, Kazem Alirezalu.

Visualization: Hasan Ahmad.

Writing-original draft: Hasan Ahmad.

Writing-review & editing: Sodeif Azadmard-Damirchi, Kazem Alirezalu.

Conflict of Interests

None.

Ethical Issues

This study did not involve human participants or animals; therefore, ethical approval was not required.

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