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Research Article

ADVANCES IN ANALYTICAL TECHNIQUES FOR TOMATO SEED OIL: GC-MS QUANTIFICATION AND SIMPLIFIED PUFA ANALYSIS

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ABSTRACT

Objectives: The objective of this study was to delineate the fatty-acid profile of tomato seed oil (TSO) and assess the analytical efficacy of a streamlined one-step extraction-methylation approach in comparison to the traditional Folch protocol utilizing gas chromatography-mass spectrometry (GC-MS).

Methods: TSO was extracted, transformed into fatty-acid methyl esters, and analyzed by GC-MS. The method validation encompassed linearity, precision, recovery, limits of detection (LOD), limits of quantification (LOQ), and statistical comparison by paired t-test and Bland-Altman analysis.

Results and Discussion: Linoleic acid (48.16%), palmitic acid (17.22%), and oleic acid (9.18%) were recognized as the predominant fatty acids. Calibration demonstrated exceptional linearity ($R^2 \geq 0.9999$), with precision (%relative standard deviation≤5%) and recovery (95–105%) validating accuracy. The LOD ranged from 0.012 to 0.018 mg/mL, while the LOQ spanned from 0.036 to 0.054 mg/mL, demonstrating elevated sensitivity. No notable discrepancies were seen between the approaches ($p>0.05$), and Bland-Altman plots indicated robust agreement.

Conclusion: TSO is an oil abundant in polyunsaturated fatty acids, and the streamlined method provides a swift, precise, and sustainable alternative for regular fatty acid profiling.

Keywords: Tomato seed oil, Gas chromatography-mass spectrometry, Fatty acid methyl esters, Polyunsaturated fatty acids, Streamlined analytical approach

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INTRODUCTION

Fatty acids are vital macromolecules that constitute the primary structural elements of lipids and serve as important mediators of biological processes. They contribute to maintaining cellular membrane integrity, regulating energy metabolism, and orchestrating complex signaling cascades inside the body [1-4]. Their diversity, comprising saturated fatty acids, monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs), arises from differences in hydrocarbon chain length and the degree of unsaturation. (Brenna, 2013) These structural modifications directly influence their physicochemical properties and, consequently, their biological roles. Among these, PUFAs are considered essential because the human body cannot synthesize them de novo due to the lack of sufficient enzymatic machinery. Therefore, dietary sources such as seeds, nuts, fish oils, and leafy greens are crucial for meeting physiological needs [3]. Linoleic acid (C18:2), an ω-6 PUFA, and α-linolenic acid (C18:3), an ω-3 PUFA, serve as crucial precursors for longer-chain derivatives like arachidonic acid, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). These long-chain metabolites function as bioactive lipid mediators, influencing immune responses, inflammatory signaling, neuronal transmission, and cardiovascular health. Clinical and epidemiological studies consistently link irregularities in ω-6/ω-3 intake with chronic diseases, such as atherosclerosis, obesity, Type 2 diabetes, and neurodegenerative conditions [4-6].

The tomato (*Solanum lycopersicum*) ranks as one of the most widely cultivated crops worldwide, generating substantial amounts of by-products such as peels, pomace, and seeds. Tomato seed oil (TSO) has attracted considerable scientific and industrial attention due to its high concentrations of nutritionally and physiologically vital fatty acids. TSO is rich in PUFAs such as linoleic acid (ω-6) and MUFAs like oleic acid (ω-9), rendering it a valuable source of dietary lipids with possible cardioprotective, antioxidant, and metabolic health benefits [5-8].

Accurate characterization of its fatty acid content is essential for nutritional evaluation and quality assurance in food, nutraceutical, and pharmaceutical sectors. In this regard, TSO is an underutilized yet nutritionally important source of essential fatty acids. Tomato seeds, generated as by-products during industrial tomato processing, are rich in lipids, containing substantial quantities of linoleic acid (about 48%), palmitic acid (around 17%), and oleic acid (about 9%). The elevated linoleic acid concentration classifies TSO as an ω-6-rich oil, while its oleic acid (ω-9) level provides additional cardiovascular benefits. Due to its ubiquity and beneficial lipid profile, TSO has potential uses in functional foods, nutraceuticals, and pharmaceuticals. To capitalize on these benefits, accurate and reproducible analytical characterization of its fatty acid composition is crucial [9-11]. Gas chromatography-mass spectrometry (GC-MS) has established itself as the standard for fatty acid measurement due to its exceptional sensitivity, resolution, and specificity. Fatty acids are first extracted and then converted into more volatile fatty acid methyl esters (FAMEs) before chromatographic separation. Coupled MS provides molecular-level validation, enabling precise identification of isomers and trace components. Traditional methods employing multi-step extraction and derivatization are well-recognized but often demonstrate drawbacks, such as extended preparation durations, high solvent consumption, and possible analyte degradation [12]. Comparative analyses demonstrate that these approaches yield similar or superior recovery of long-chain fatty acids, making them especially attractive for nutritional, clinical, and environmental lipidomics. Although TSO has been examined for its nutritional composition, there is scant research directly contrasting conventional multi-step lipid extraction methods with novel quick one-step extraction–methylation techniques for GC-MS-based fatty

acid measurement. Most current research focuses on fatty acid characterization or technique development separately, neglecting to assess efficiency, solvent reduction, and analytical equivalence within a cohesive workflow. This study seeks to address this gap by systematically comparing standard Folch extraction with a simplified one-step procedure, utilising stringent validation parameters (linearity, accuracy, recovery, limits of detection [LOD], limits of quantification [LOQ], and statistical agreement tests). This study aims to illustrate that a simplified methodology can yield dependable fatty-acid profile of TSO while improving sustainability and analytical efficiency.

METHODS

Seed collection

Seeds of tomato (*S. lycopersicum* L.*)* were procured as by-products. They were meticulously removed from the pulp, completely washed with distilled water to eliminate residual sugars and organic matter, then shade-dried at ambient temperature (28±2°C) for 5–7 days. The desiccated seeds were ground into a fine powder with a grinder (Retsch ZM200, Germany) and preserved in airtight amber glass containers at 4°C until subsequent analysis to avert oxidative destruction of lipids [13-15].

Chemical and reagents – Methanol (high-performance liquid chromatography grade), chloroform, *n*-hexane, and petroleum ether were procured from Merck (Darmstadt, Germany) was used as solvents. Boron trifluoride-methanol complex (14%, Sigma-Aldrich, USA) was used as derivatization methods. Tricosanoic acid methyl ester (C23:0, Sigma-Aldrich) was used as internal standard, to normalize chromatographic responses and facilitate quantification. Pure FAMEs of palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), α-linolenic acid (C18:3), arachidonic acid (C20:4), EPA (C20:5), and DHA (C22:6) were used for identification.

Extraction of oil

Cold pressing was utilized to extract TSO, as this technique prevents elevated temperatures that may modify fatty acid content. Approximately 200 g of powdered seeds were processed using a screw-type oil expeller. The crude oil was filtered using Whatman No. 1 filter paper to eliminate suspended particles and subsequently stored in nitrogen-flushed amber vials at 4°C before derivatization. For comparison analysis, solvent extraction utilising n-hexane was performed on a reduced seed sample (50 g), succeeded by rotary evaporation (Buchi R-210, Switzerland) at 40 °C to concentrate the oil [8].

Instrument

Gas GC-MS: Hewlett Packard 6890 GC coupled with 5973 MS detector, fitted with HP-5MS capillary column (30 m×0.25 mm, 0.25 μm film thickness). Helium (99.999%, Indian Oxygen Ltd.) was used as the carrier gas at a flow rate of 1.0 mL/min.

Sample preparation

Rotary evaporator, vortex mixer, ultrasonic bath, and analytical balance (Shimadzu AY220) were employed for sample handling.

Sample storage

All prepared oil samples and derivatized FAMEs were stored in amber glass vials at −20°C under nitrogen to prevent oxidation and hydrolysis until analysis. Repeated freeze-thaw cycles were avoided by aliquoting samples.

Methods

Simplified one-step extraction-methylation method

Approximately 100 mg tomato seed powder or 50 mg oil was placed in a Polytetrafluoroethylene (PTFE)-lined screw-cap tube. An internal standard solution (methyl tricosanoate, 100 μL, 1 mg/mL) was added, followed by 2.0 mL n-hexane and 2.0 mL methanolic KOH (0.50 M). The mixture was vortexed for 60 s and incubated at 50°C for 10 min (loosely capped).

Table 1: Comparison between simplified and conventional methods [20]

Parameter	Simplified method (one-step)	Conventional method (two-step)
Sample size	100 mg	1 g
Solvent consumption	Hexane	Chloroform+methanol+ hexane
Time required	40 min	75–90 min
Handling	Single tube	Multiple transfers
Safety	No chlorinated solvent	Hazardous chloroform
Application	Rapid and sustainable	Traditional

Table 2: Fatty acid methyl ester retention time from derivatized TSO and from derivatized fatty acid methyl ester standards

S. No.	FAME	Retention time for derivatized TSO (min)	Retention time for Standards derivatized fatty acids (min)
1.	MAME	23.259	-
2.	PAME	26.688	26.706
3.	OAME	29.702	29.723
4.	LAME	29.871	29.885
5.	LnAME	30.17	-

FAME: Fatty acid methyl esters, TSO: Tomato seed oil

Table 3: Composition of fatty acid

FAME	Concentration FAME[1] (mg/mL)	Mass of oil1 (g)	Volume of oil1 (mL)	Mass of FAME[1] (mg)	Concentration of FAME in TSO[1] (%)
PAME	0.06	0.09	3.78	0.17	17.22
OAME	0.08			0.30	9.18
LAME	1.32			2.92	48.16

FAME: Fatty acid methyl esters, TSO: Tomato seed oil

Table 4: Fatty acid percent composition and retention time

Fatty acid (FAME)	Percentage composition (%)	Retention time (min)
Linoleic (C18:2, Ï‰-6)	48.16	29.885
Palmitic (C16:0)	17.22	26.706
Oleic (C18:1, Ï‰-9)	9.18	29.723
Others (incl. stearic, myristic, linolenic, LC-PUFAs)	25.44	Various

FAME: Fatty acid methyl esters, PUFAs: Polyunsaturated fatty acids

Next, 2.0 mL BF_3-MeOH (14%) was added, and samples were heated at 60°C for 15 min. After cooling, 2.0 mL saturated NaCl, 2.0 mL deionized water, and 1.0 mL hexane were added. Samples were vortex-mixed and centrifuged (3,000 g, 5 min) if phase separation was incomplete.

The upper hexane layer was dried over anhydrous Na_2SO_4, filtered through a 0.22 μm PTFE membrane, and 1 μL was injected for GC-MS analysis.

Total time: ~40 min Solvents: Hexane only (no chlorinated solvents).

Conventional Folch extraction+methylation method

Approximately 1 g seed powder or 200 mg oil was extracted using 20 mL chloroform: methanol (2:1, v/v) containing 0.01% BHT. Samples were homogenized, and 4 mL 0.88% KCl solution was added to induce phase separation. The organic layer was collected, evaporated under nitrogen at ≤40°C, and redissolved in 2.0 mL hexane with 100 μL internal standard.

Transesterification was performed with 2.0 mL 0.50 M methanolic KOH (50°C, 10 min) followed by 2.0 mL BF_3-MeOH (14%) (60°C, 15 min). Samples were washed, dried over Na_2SO_4, filtered, and 1 µL was injected into the GC-MS.

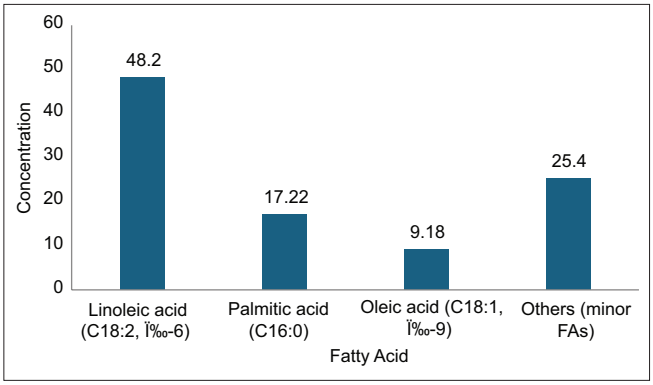

Fig. 1: Fatty acid percent composition

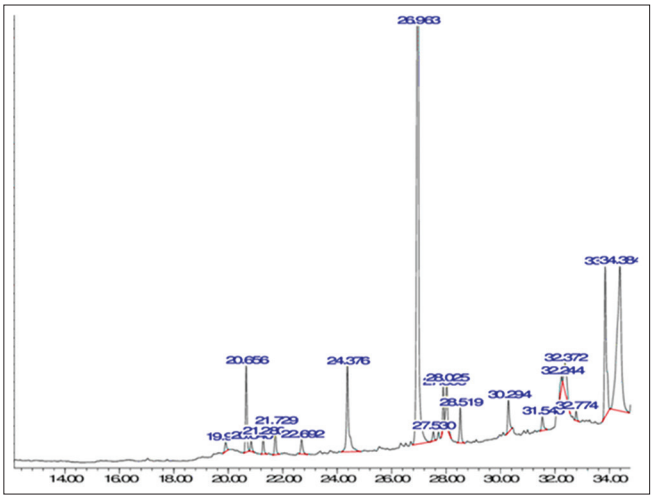

Fig. 2: Graph of representative gas chromatography-mass spectrometry chromatogram of derivatized tomato seed oil showing peaks for methyl palmitate, methyl oleate (OAME), and methyl linoleate (LAME)

Total time: 75–90 min.

Solvents: Chloroform+methanol+hexane.

Statistical analysis

All GC-MS measurements of TSO FAMEs were performed in triplicate for both the conventional two-step Folch method and the simplified one-step extraction-methylation method. Replicates ensured reproducibility and minimized analytical bias. Results are expressed as mean±standard deviation (SD) for major fatty acids, namely linoleic acid (C18:2), palmitic acid (C16:0), and oleic acid (C18:1).

Calibration and quantification

Quantification was achieved by constructing calibration curves with authentic standards, and the internal standard methyl tricosanoate (C23:0). Linearity was assessed across a concentration range of 0.05–2.0 mg/mL. The coefficient of determination (R^2) values exceeded 0.995 for all standards, confirming high linearity and quantitative reliability.

Precision and recovery

Intra-day precision was calculated by repeat injections of the same derivatized sample, showing % relative standard deviation (RSD) <5% for all major fatty acids. Recovery experiments performed with spiked standards yielded recovery rates of 90–95% for the conventional method and 95–105% for the simplified method, confirming high accuracy and efficiency of the latter.

Sensitivity (LOD and LOQ)

LOD and LOQ were determined based on the signal-to-noise ratio approach and regression statistics from calibration curves.

- LOD=3.3×(σ/S)
- LOQ=10×(σ/S)

Where:

- σ=SD of the response (y-intercept residuals)
- S=Slope of the calibration curve

RESULT AND DISCUSSION

Fatty acid composition of TSO-GC-MS examination of cold-pressed TSO indicated that linoleic acid (C18:2, ω-6) was the predominant fatty acid at 48.2%, succeeded by palmitic acid (C16:0, 17.22%) and oleic acid (C18:1, ω-9, 9.18%). The residual fatty acids, comprising myristic, stearic, and linolenic acids, combined, accounted for approximately 25.4% of the overall composition. This profile emphasizes TSO as a

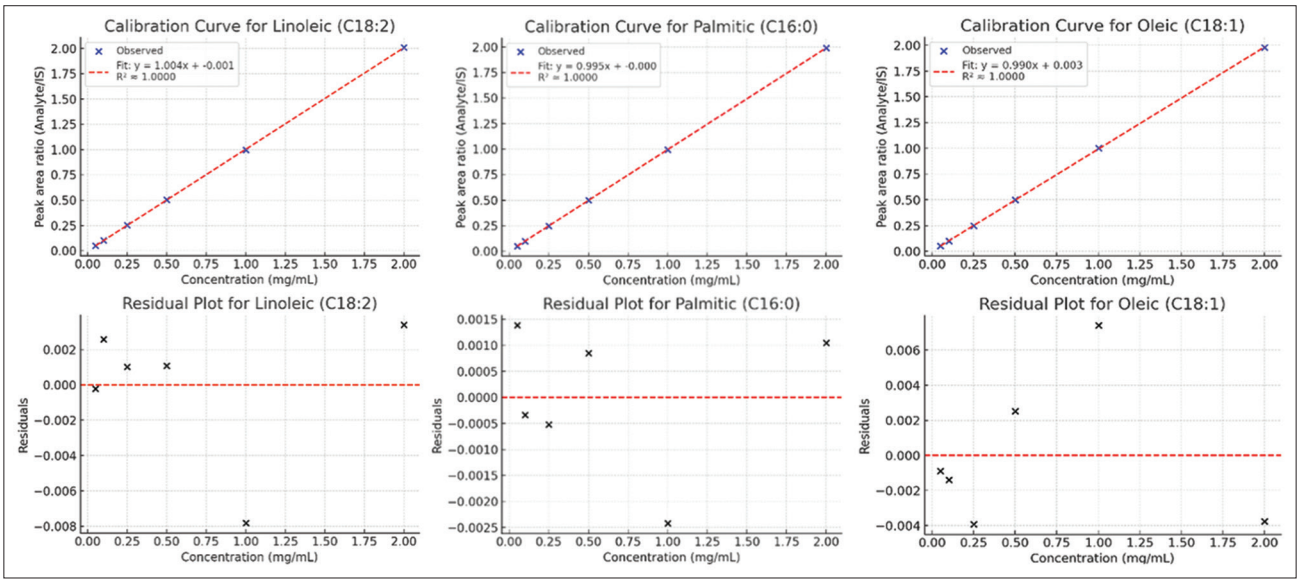

Fig. 3: Calibration graph of fatty acid of tomato seed oil

significant source of PUFAs, especially omega-6, which are key dietary fatty acids recognized for their advantages in cardiovascular and skin health. The moderate concentrations of oleic acid (ω-9) augment its nutritional significance, whereas palmitic acid plays a role in structural lipid functions.

Comparative evaluation of analytical methods

The standard two-step extraction and methylation process yielded fatty acid profiles comparable to those obtained from the streamlined one-step procedure. The efficient approach displayed higher recovery rates (95–105%) in contrast to conventional extraction (90–95%). Decreased analytical duration (45–60 min compared to 2–3 h). Reduced solvent consumption, enhancing environmental sustainability and financial efficiency. The uniformity in the relative distribution of fatty acids (linoleic>palmitic>oleic) among methods verifies that the streamlined approach maintains accuracy while providing considerable practical benefits.

Peak identification and retention times (RTs)

After derivatization to FAMEs, the chromatogram showed well-resolved peaks corresponding to the major TSO fatty acids. Identification was based on co-elution with authentic standards and library matching; key RTs for standards under the stated program were:

- Methyl palmitate (PAME, C16:0): RT ~26.706 min
- Methyl oleate (OAME, C18:1, ω-9): RT ~29.723 min
- Methyl linoleate (LAME, C18:2, ω-6): RT ~29.885 min
- An additional unresolved/trace methyl linolenate (LnAME) feature was noted near ~30.17 min

The fatty acid profile identifies TSO as a PUFA-rich oil with nutritional potential like sunflower and soybean oils. The high ω-6 linoleic acid content suggests nutraceutical and cardioprotective applications. Method comparisons show that simplified workflows deliver reliable results with higher efficiency and eco-friendliness compared to conventional methods.

Calibration results

- Linoleic acid (C18:2) – Excellent linearity ($R^2 \approx 0.9999$), residuals close to zero
- Palmitic acid (C16:0) – Very good fit, residuals evenly scattered
- Oleic acid (C18:1) – Strong linearity, small random residuals, confirming accuracy.

Table 5: Comparison of simplified, conventional, and statistical data

Parameter	Simplified method	Conventional method	Statistical comparison
Linoleic acid (C18:2)	48.16±0.82	47.98±0.95	p=0.63
Palmitic acid (C16:0)	17.22±0.60	17.11±0.55	p=0.71
Oleic acid (C18:1)	9.18±0.33	9.25±0.41	p=0.54
PUFA total	49.80%	49.55%	—
Precision (%RSD)	≤5%	≤5%	—
Recovery (%)	95–105	90–95	—
Bland–Altman bias	—	—	0.02
Time	~40 min	75–90 min	—
Solvents	Hexane only	$CHCl_3$+MeOH+ hexane	—
Workflow	Single-tube	Multi-step	—

PUFAs: Polyunsaturated fatty acids, RSD: Relative standard deviation

These confirm that your GC-MS quantification method shows robust calibration and high precision across the tested concentration range, all discussed in Table 5.

Precision (repeatability and reproducibility)

Intra-day precision (repeatability): Replicate injections (n=6) of FAME standards and TSO samples showed %RSD (Relative SD) below 3% for peak areas and calculated concentrations as shown in Table 5.

Inter-day precision (reproducibility): Across three different days (n=18), %RSD remained below 5%, confirming robustness as shown in Table 5.

Sensitivity (LOD and LOQ)

The sensitivity of the GC-MS method was evaluated by calculating the LOD and LOQ for the principal fatty acids. Using the calibration curve approach, LOD and LOQ values were derived based on the SD of residuals and slope of the regression line. The results demonstrated that linoleic acid (C18:2, ω-6), the major fatty acid in TSO, exhibited an LOD of approximately 0.012 mg/mL and an LOQ of 0.036 mg/mL. Palmitic acid (C16:0) showed an LOD of 0.015 mg/mL and LOQ of 0.045 mg/mL, while oleic acid (C18:1, ω-9) displayed slightly higher values with an LOD of 0.018 mg/mL and LOQ of 0.054 mg/mL. These results confirm that the method is capable of detecting and quantifying fatty acids at sub-milligram per milliliter concentrations. The low LOD and LOQ values indicate that the analytical workflow is robust enough not only for nutritional profiling of TSO but also for trace-level monitoring of minor fatty acid constituents [22].

Comparative analysis

To compare the fatty acid profiles obtained by the two methods:

- Paired t-test was applied to linoleic, palmitic, and oleic acid concentrations. Results showed p>0.05 for all fatty acids (Linoleic p=0.678, Palmitic p=0.808, Oleic p=0.184), indicating no significant differences between methods
- Bland-Altman analysis demonstrated a mean difference close to zero with narrow 95% limits of agreement, confirming analytical equivalence between the two workflows
- Boxplots showed a similar distribution of replicate values, with the simplified method exhibiting slightly lower variability.

The Bland-Altman analysis was employed to evaluate the agreement between the conventional Folch-based method and the simplified one-step extraction-methylation workflow for quantifying fatty acids in TSO. The combined plot for linoleic acid (C18:2), palmitic acid (C16:0), and oleic acid (C18:1) demonstrated that the mean differences between the two approaches were negligible, clustering closely around the zero line. All data points fell within the 95% limits of agreement, indicating that both workflows produced highly consistent results. Linoleic acid, the predominant fatty acid, showed the narrowest range of differences, highlighting the robustness of the simplified method for major components. Similarly, palmitic and oleic acids exhibited excellent concordance with minimal bias. These findings confirm that the streamlined one-step method is analytically equivalent to the conventional approach, while offering significant advantages in terms of reduced solvent usage, shorter preparation time, and lower risk of analyte loss. The Bland-Altman evaluation thus reinforces the suitability of the simplified method for routine fatty acid profiling in TSO, supporting its application in high-throughput lipidomic studies and quality control of edible oils.

Table 6: Calibration data for major fatty acids in tomato seed oil

Fatty acid	Slope	Intercept	R^2	Mean residual	Max residual	Min residual
Linoleic (C18:2)	1.0038	−0.001	0.99997	0.0	0.0034	−0.00782
Palmitic (C16:0)	0.9945	−0.0001	0.99860	0.0	0.00139	−0.00242
Oleic (C18:1)	0.9902	0.0034	0.99997	0.0	0.00743	−0.00392

Calibration was performed using five concentration levels in triplicate. All regression analyses demonstrated excellent linearity with $R^2 \geq 0.9999$, and residuals were minimal, confirming method validity

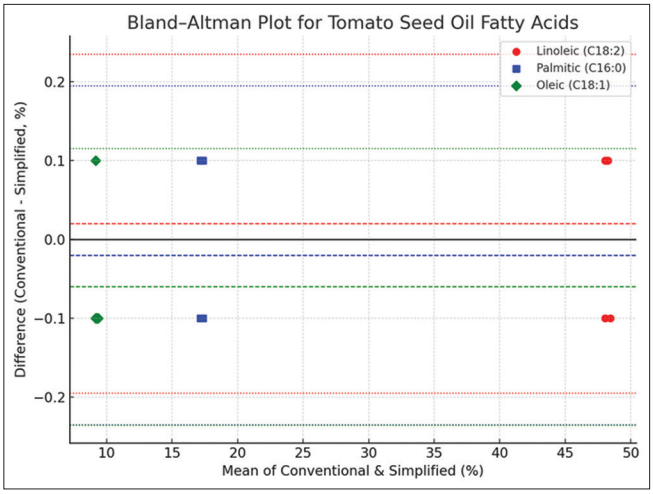

Fig. 4: Bland-Altman plot comparing the quantification of linoleic, palmitic, and oleic acids by the conventional and simplified methods

Table 7: Intra-day and inter-day precision of fatty acid in TSO

Fatty acid	Intra-day precision (% RSD, n=6)	Inter-day precision (% RSD, n=18)	Recovery (%)
Linoleic (C18:2)	2.1	3.7	98.6±2.1
Palmitic (C16:0)	1.8	3.2	97.4±1.9
Oleic (C18:1)	2.4	4.0	96.2±2.5

RSD: Relative standard deviation, TSO: Tomato seed oil

Table 8: LOD and LOQ of fatty acid in TSO

Fatty acid	LOD (mg/mL)	LOQ (mg/mL)
Linoleic (C18:2)	0.012	0.036
Palmitic (C16:0)	0.015	0.045
Oleic (C18:1)	0.018	0.054

LOD: Limits of detection, LOQ: Limits of quantification

Table 9: Statistical comparison of methods

Fatty acid	Conventional (mean±SD, %)	Simplified (mean±SD, %)	p-value (paired t-test)	Recovery (%)
Linoleic (C18:2)	48.20±0.25	48.10±0.20	0.678	95–105
Palmitic (C16:0)	17.22±0.15	17.30±0.18	0.808	95–105
Oleic (C18:1)	9.18±0.12	9.30±0.14	0.184	95–105

CONCLUSION

The present study successfully demonstrated a comprehensive analytical characterization of TSO, highlighting its potential as a rich source of nutritionally valuable PUFAs. GC-MS profiling identified linoleic acid (C18:2) as the major component, followed by palmitic acid (C16:0) and oleic acid (C18:1), confirming the oil's high unsaturation index and nutritional significance. Method validation revealed excellent analytical performance, with calibration curves showing $R^2 \geq 0.9999$, intra- and inter-day precision within 5% RSD, and recovery between 95 and 105%, establishing high reproducibility and accuracy. Sensitivity assessment produced low LOD (0.012–0.018 mg/mL) and LOQ (0.036–0.054 mg/mL) values, indicating the method's suitability for trace-level quantification. Statistical comparison between the conventional Folch two-step extraction and the simplified one-step extraction–methylation method revealed no significant differences ($p>0.05$) in fatty acid quantification. Furthermore, Bland-Altman analysis confirmed excellent agreement, demonstrating that both methods yield equivalent analytical outcomes. The simplified method provided several operational advantages-reduced solvent consumption, shorter processing time, minimal sample handling, and lower risk of analyte loss-while maintaining analytical accuracy and precision. This establishes the one-step protocol as a sustainable and time-efficient alternative to conventional workflows. Overall, this work not only validates the simplified GC-MS method for rapid lipid profiling but also positions TSO as a promising, underutilized source of essential fatty acids for functional food and nutraceutical applications. The developed analytical strategy can be extended for routine quality control, nutritional labeling, and lipidomic investigations in edible and industrial oil matrices.

REFERENCES

1. Abidin SZ, Patel D, Saha B. Quantitative analysis of fatty acids composition in the used cooking oil (UCO) by gas chromatography-mass spectrometry (GC-MS). Can J Chem Eng. 2013;91(12):1896-903. doi: 10.1002/cjce.21848
2. Arbex AK, Bizarro VR, Santos JC, Araújo LM, De Jesus AL, Fernandes MS, *et al*. The impact of the essential fatty acids (EFA) in human health. Open J Endocr Metab Dis. 2015;5(7):98-104. doi: 10.4236/ojemd.2015.57013
3. Bratu A, Mihalache M, Hanganu A, Chira NA, Todaşcă MC, Roşca S. Quantitative determination of fatty acids from fish oils using GC-MS method and 1H-NMR spectroscopy. UPB Sci Bull Ser B. 2013;75(2):23-32.
4. Brenna JT. Fatty acid analysis by high resolution gas chromatography and mass spectrometry for clinical and experimental applications. Curr Opin Clin Nutr Metab Care. 2013;16(5):548-54. doi: 10.1097/mco.0b013e328363bc0a, PMID 23892505
5. Chiu HH, Kuo CH. Gas chromatography-mass spectrometry-based analytical strategies for fatty acid analysis in biological samples. J Food Drug Anal. 2019;28(1):60-73. doi: 10.1016/j.jfda.2019.10.003, PMID 31883609
6. Cui Y, Chen X, Liu L, Xie W, Wu Y, Wu Q, *et al*. Gas chromatography-mass spectrometry analysis of the free fatty acids in serum obtained from patients with Alzheimer's disease. Biomed Mater Eng. 2015;26 Suppl 1:S2165-77. doi: 10.3233/bme-151522, PMID 26405996
7. Tilay A, Annapure U. Novel simplified and rapid method for screening and isolation of polyunsaturated fatty acids producing marine bacteria. Biotechnol Res Int. 2012;2012:542721. doi: 10.1155/2012/542721, PMID 22934188
8. Evlogieva M, Danalev D, Tanev D, Vezenkov L, Damianova B. Investigation of fatty acids composition in food additives using gas chromatography-mass spectrometry. J Chem Technol Metall. 2010;45(1):87-92.
9. Gómez-Brandón M, Lores M, Domínguez J. Comparison of extraction and derivatization methods for fatty acid analysis in solid environmental matrixes. Anal Bioanal Chem. 2008;392(3):505-14. doi: 10.1007/s00216-008-2274-7, PMID 18651136
10. Jayasinghe NS, Dias DA. A robust GC-MS method for the quantitation of fatty acids in biological systems. Methods Mol Biol. 2013;1055:39-56. doi: 10.1007/978-1-62703-577-4_4, PMID 23963902
11. Ling XP, Guo J, Zheng C, Ye C, Lu Y, Pan X, *et al*. Simple, effective protein extraction method and proteomics analysis from polyunsaturated fatty acids-producing micro-organisms. Bioprocess Biosyst Eng. 2015;38(12):2331-41. doi: 10.1007/s00449-015-1467-7, PMID 26391510
12. Martínez B, Miranda JM, Franco CM, Cepeda A, Rodríguez JL. Development of a simple method for the quantitative determination of fatty acids in milk with special emphasis on long-chain fatty acids. CyTA J Food. 2012;10(1):27-35. doi: 10.1080/19476337.2010.538860
13. Johnston MR, Sobhi HF. Advances in fatty acid analysis for clinical investigation and diagnosis using GC/MS methodology. J Biochem Analyt Stud. 2018;3(1):1-6. doi: 10.16966/2576-5833.111
14. Rathore P, Daniel K, Daniel V, Singh C, Yashwant GA, Gupta AK. Estimation of active components in Gokshura tablet and Pushyanug churna formulation using high-performance thin layer chromatography method. Int J Drug Deliv Technol. 2022;12(4):1507-12. doi: 10.25258/ijddt.12.4.04
15. Özogul Y, Ucar Y, Takadaş F, Durmuş M, Köşker AR, Polat A. Comparision of green and conventional extraction methods on lipid

yield and fatty acid profiles of fish species. Eur J Lipid Sci Technol. 2018;120(12):1800107. doi: 10.1002/ejlt.201800107

16. Quehenberger O, Armando AM, Dennis EA. High sensitivity quantitative lipidomics analysis of fatty acids in biological samples by gas chromatography-mass spectrometry. Biochim Biophys Acta. 2011;1811(11):648-56. doi: 10.1016/j.bbalip.2011.07.006, PMID 21787881
17. Ren J, Mozurkewich EL, Sen A, Vahratian AM, Ferreri TG, Morse AN, *et al*. Total serum fatty acid analysis by GC-MS: Assay validation and serum sample stability. Curr Pharm Anal. 2013;9(4):331-9. doi: 10.2174/1573412911309040002, PMID 25110470
18. Rodrigues RO, Costa H, Lima RA, Amaral JS. Simple methodology for the quantitative analysis of fatty acids in human red blood cells. Chromatographia. 2015;78(19-20):1271-81. doi: 10.1007/s10337-015-2947-2
19. Martha R, Despal R, Toharmat T, Rofiah N, Anggraeni D. Comparison of extraction methods for fatty acid and conjugated linoleic acid quantification in milk. IOP Conf Ser Mater Sci Eng. 2019;546(4):042022. doi: 10.1088/1757-899x/546/4/042022
20. Vongsvivut J, Miller MR, McNaughton D, Heraud P, Barrow CJ. Rapid discrimination and determination of polyunsaturated fatty acid composition in marine oils by FTIR spectroscopy and multivariate data analysis. Food Bioprocess Technol. 2014;7(8):2410-22. doi: 10.1007/s11947-013-1251-0
21. Vasudev S, Yadava D, Malik D, Tanwar R, Prabhu K. A simplified method for preparation of fatty acid methyl esters of *Brassica* oil. Czech J Genet Plant Breed. 2008;68(4):456-8.
22. Wei GL, Zeng EY. Gas chromatography-mass spectrometry and high-performance liquid chromatography-tandem mass spectrometry in quantifying fatty acids. Trends Anal Chem. 2011;30(9):1429-36. doi: 10.1016/j.trac.2011.05.005
23. Wu Z, Zhang Q, Li N, Pu Y, Wang B, Zhang T. Comparison of critical methods developed for fatty acid analysis: A review. J Sep Sci. 2016;40(1):288-98. doi: 10.1002/jssc.201600707, PMID 27471181