

Research Article

Comparison of Extraction Techniques on Dried Lavender Flowers Using Gas Chromatography-Mass Spectrometry

Nurhidayah Basri, Geraldine Chan, Panita Ngamchuachit* and Aaron Thong*

Singapore Institute of Food and Biotechnology Innovation (SIFBI), Agency for Science, Technology and Research (A*STAR), Nanos, Singapore

* Corresponding author. E-mail: Panita_Ngamchuachit@a-star.edu.sg; aaron_thong@a-star.edu.sg

DOI: 10.14416/j.asep.2026.05.010

Received: 25 November 2025; Revised: 4 March 2026; Accepted: 20 April 2026; Published online: 28 May 2026

© 2026 King Mongkut's University of Technology North Bangkok. All Rights Reserved.

Abstract

Lavender essential oil is widely used in perfumery, cosmetics, aromatherapy, and food applications, with quality largely determined by the composition of key monoterpenoids. Common lavender (*Lavandula angustifolia*) is regarded as the reference species for ISO-compliant oils due to its characteristic aroma profile and low camphor content. This study compared steam distillation (SD), simultaneous distillation–extraction (SDE), and solvent-assisted flavor evaporation (SAFE) to investigate their effects on the volatile composition of dried *L. angustifolia* flowers. Volatiles were analyzed by gas chromatography–mass spectrometry (GC-MS), and four key odorants (linalool, linalyl acetate, lavandulol, and lavandulyl acetate) were quantified by gas chromatography–flame ionization detection (GC-FID). A total of 30 compounds were identified using spectral and retention index matches to an in-house library and the NIST 17 library. Two-way ANOVA revealed significant differences among extraction methods (p -value < 0.05). SD yielded the highest concentrations of linalool and linalyl acetate, consistent with ISO 3515:2002 specifications, whereas SAFE produced the lowest amounts but the most diverse volatile profile, including more thermally labile and minor constituents than SD or SDE. These results highlight a clear trade-off: SD maximizes yields of ISO-defined volatiles, whereas SAFE better reflects aroma authenticity, offering complementary insights for lavender quality assessment from a sensomics perspective.

Keywords: Extraction, GC-MS, Lavender, Solvent-assisted flavor evaporation (SAFE), Volatile compounds

1 Introduction

Lavender species of the genus *Lavandula* belong to the Lamiaceae family. This family includes over 30 species and almost 400 registered cultivars [1]. Although *Lavandula* species are native to the Mediterranean area, it is cultivated worldwide for their essential oils (EO), which are commonly used in aromatherapy, perfumery, personal care, and food products. The three main commercial species for the production of lavender EO are: common lavender (*L. angustifolia*), spike lavender (*L. latifolia*) and lavandin (*L. intermedia*) - a hybrid of *L. angustifolia* x *L. latifolia* [2].

True lavender (*L. angustifolia*) EO is more expensive than the other lavender species due to its high quality and low oil yield of only 2–5%. Lavandin

cultivars, on the other hand, are more robust and have a yield of 3 times more oil than lavender. As a result, world production of lavender is now mainly focused on lavandin [3]. World production of lavender EO is about 200 tons per annum, while lavandin EO is significantly higher at 1200 tons per annum [4], [5]. Lavender EOs are sometimes misidentified or marketed as spike lavender or lavandin oils [6]. Volatile components of these oils are dependent on plant chemotype, geographic region of origin, harvest conditions and post-harvest treatments like storage and drying [7], [8]. The key difference between the EOs is the low amount of camphor in lavender EO (less than 1%), while lavandin EO contains about 10–12% camphor [4], [9]. Lavender EO is extracted by steam distillation (SD) of *L. angustifolia* leaves and flowers [5]. The quality and composition of the EO is

influenced by distillation time. Short distillation times yield oils that are missing compounds with higher boiling points. However, longer distillation times may yield oils with unpleasant smells [4]. The primary volatile components of lavender EO are monoterpenoids like linalool and linalyl acetate [10]–[12]. Based on the European Pharmacopoeia's reference value, lavender EO must contain between 20–45% linalool, 25–46% linalyl acetate and less than 1.2% camphor [3]. In higher quality lavender EO, the ratio between linalyl acetate and linalool should be greater than 1 [4]. Almost 75% of the total EO yield is obtained within the first 25 minutes of distillation to produce a commercial-grade lavender EO. Today, some industrial distillation processes operate at high temperature and pressure to shorten extraction time, which may affect oil quality. In certain cases, synthetic aroma compounds such as linalyl acetate may be added to adjust fragrance characteristics [13], [14].

Aside from steam distillation (SD), there are several other methods used to extract the volatile components of food or plant matrices which have been reported in literature such as: Simultaneous distillation extraction (SDE) [15], solid-phase micro extraction (SPME) [16], solvent-assisted flavor evaporation (SAFE) [17], supercritical CO₂ (SFE) and ultrasound-assisted extraction (USE) [18]. SDE combines both steam distillation and liquid-liquid extraction using a volatile solvent. Since both SD and SDE are carried out at elevated temperatures, this may also lead to the formation of artefacts such as esters and acetals [19]–[21], which may not necessarily be viewed negatively in perfumery [22]. SPME is a solvent-free extraction technique that utilizes a piece of fused silica coated with a liquid or solid phase to extract volatile components [23]. This technique is simple, fast [24] and selective to the types of target aroma compounds based on the material of the coating. SAFE is a versatile technique to extract volatile aroma components from food matrices in both aqueous matrices and solvent extracts [17]. Moreover, SAFE is performed at a low temperature (about 40 °C) and high vacuum (5×10^{-3} Pa), hence preventing the formation of artefacts as well as preserving thermally unstable compounds [25], [26]. Recent studies have combined advanced analytical platforms such as GC–IMS and GC–MS with multivariate analysis to comprehensively characterize volatile organic compounds in lavender EOs, enabling improved differentiation of chemotypes and aroma characteristics [10]. However, regardless of analytical

sophistication, the representativeness of the aroma profile fundamentally depends on the extraction technique, highlighting the importance of gentle approaches such as SAFE for preserving thermally labile and low-abundance odorants.

The aim of this work is to evaluate how three different extraction techniques would influence the volatile composition of dried lavender flowers and to assess their respective advantages and limitations. Comparative analysis of SD, SDE and SPME for lavender has already been reported in literature [11],[27]. Although SD and SDE have been previously reported for lavender, SD is the industry reference method for ISO-compliant EO, while SDE provides improved recovery of semi-volatile compounds, allowing comparison with existing literature. However, both techniques involve high temperatures that may induce thermal degradation or artefact formation. SAFE, a high-vacuum, low-temperature method, has not been widely reported for dried lavender and is expected to better preserve thermally labile volatiles. Therefore, SD and SDE were included as benchmark techniques, and SAFE was selected to evaluate whether a gentler extraction yields a more representative aroma profile.

2 Materials and Methods

2.1 Samples

A 250 g dried lavender flowers (*L. angustifolia*) were commercially purchased from Phoon Huat Pte Ltd (Singapore). The sample was stored in a sealed bag at -20 °C prior to use. Lavender EOs (L1 and L2) were obtained from Thursday Plantation (USA) and Nila Singapore Pte Ltd (Singapore), respectively. Lavandin EOs, LN1 and LN2 were obtained from Nila Singapore Pte Ltd (Singapore).

2.2 Chemicals and reagents

Anhydrous diethyl ether (> 99.00 %, AR grade) was purchased from VWR (Singapore), pentane (HPLC grade) and hexane (> 95.0 %, HPLC certified) were purchased from Tee Hai Chem (Singapore), C7-C30 saturated alkanes standard and 1-undecanol were purchased from Sigma Aldrich (USA). Ultrapure water was obtained from a Milli-Q water purification system (Sartorius, Germany). Anhydrous sodium sulphate was purchased from GCE Lab Chemicals (Singapore). Pentane-diethyl ether (1:1.12) mixture was distilled before use. The Likens-Nickerson

apparatus was obtained from UFO Labglass (Singapore), the SAFE apparatus from Glasbläserei Bahr (Germany). Where possible, all glassware was washed and pyrolyzed in a pyrolysis oven (Jin Gang, Singapore) at 450 °C for at least 6 hours prior to use.

2.3 Sample preparation

2.3.1 Moisture analysis

The moisture content of dried flowers (1 g) was determined in triplicate using an HS153 moisture analyzer (Mettler Toledo, Ohio, USA) in triplicates.

2.3.2 Sample S1: Steam Distillation (SD)

Ultrapure water (700 mL) was added to a 1 L distillation boiling flask, and a 50 g portion of lavender flowers was placed in a charging flask above it. The flask was connected to a connection catheter, which was subsequently connected to a condenser and a constant pressure separator receiver. The flask was heated to boiling by an electric heater for 2 h and then allowed to cool for 45–60 min. Two layers were observed in the receiver, consisting of hydrosol (bottom) and lavender EO (top). The fractions were collected separately. Anhydrous sodium sulphate was added to the organic fraction and then filtered through hydrophobic poly-tetrafluoroethylene (PTFE) syringe filters (13 mm, 0.22 µm) (Thermo Scientific, China). The samples were subsequently sealed and stored at 4 °C prior to analysis.

2.3.3 Sample S2: Simultaneous Distillation Extraction (SDE)

SDE was performed using a Likens-Nickerson apparatus [15]. A 25 g portion of dried flowers and 200 mL ultrapure water were added to a 1 L round-bottom flask, while 12.5 mL distilled pentane-diethyl ether mixture (1:1.12) was added to a 50 mL round-bottom flask. The circulating water temperature was maintained at 4 °C. The flask with dried flowers and water mixture was heated to boiling by an electric heater, while the flask with solvent was heated over a 50 °C water bath. The biomaterial was extracted for 1 h, after which the apparatus was cooled. The collected solvent extracts were dried with anhydrous sodium sulphate and filtered using Whatman™ Grade 1 cellulose filter paper (Cytiva, USA) and concentrated to 1 mL using a Vigreux distillation column. The extract was stored at 4 °C prior to analysis.

2.3.4 Sample S3: Solvent Assisted Flavor Extraction (SAFE)

A 15 g portion of dried lavender flowers was flash frozen in liquid nitrogen, ground, and stirred with 100 mL distilled pentane-diethyl ether (1:1.12) for 1 hour. The mixture was filtered using Whatman™ Grade 1 cellulose filter paper (Cytiva, USA), and the residue was re-extracted twice with 100 mL of solvent. The combined filtrates were dehydrated using anhydrous sodium sulphate, filtered, and subjected to SAFE distillation to obtain the volatile fraction [17]. During the process, the water bath temperature was maintained at 30 °C and the circulating jacket at 20 °C. The pressure was reduced to approximately 10⁻⁶ mbar, and the volatiles were collected in a liquid-nitrogen-cooled flask. The extract was then thawed, concentrated to 1 mL using a Vigreux column, and stored at 4 °C until analysis.

2.4 GC-MS and GC-FID analysis

GC-MS analysis was carried out using an Agilent 7890B GC System coupled to a 5977B mass spectrometer (MS) (Agilent Technologies, Santa Clara, CA, USA) equipped with a multimode inlet (Gerstel GmbH & Co.KG, Germany). Separation was achieved on a non-polar J&W HP-5 MS UI capillary column (30 m x 0.25 mm x 0.25 µm) (Agilent Technologies, Santa Clara, CA, USA). The stationary phase for the column is (5%-phenyl)-methylpolysiloxane, with low bleed characteristics. A straight ultra-inert inlet liner with glass wool (Agilent Technologies, Santa Clara, CA, USA) was used. The split ratio was 1:300 with an injection volume of 1 µL. Analyses were carried out in triplicate. Samples S1, L1, and L2 were diluted 100-fold, while S2 and S3 were diluted 10-fold in hexane. The oven temperature program was 50 °C (3 °C/min) to 180 °C (10 °C/min) and then held at 270 °C for 10 minutes. Helium carrier gas was set at a constant flow of 1.0 mL/min. The MS was operated in electron ionization mode at 70 eV, with a scan range of m/z 50 to 450 in full scan mode and an acquisition rate of 3.6 scans/s. The source and transfer line temperatures were 230 °C and 250 °C, respectively. Methanol and hexane were used for blank runs and needle washing.

GC-FID analysis was carried out using an Agilent 7890A GC system equipped with a flame ionization detector (FID) and a split/splitless injector (Agilent Technologies, CA, USA). The column and oven program were identical to those used for GC–

MS. The split ratio was 1:100 with an injection volume of 1 μ L. Analyses were performed in triplicate. Carrier gas flow (helium) was 1.0 mL/min. Hydrogen, air, and make-up gas flow rates were 30, 400, and 25 mL/min, respectively.

2.5 Data processing analysis

Volatile compounds were putatively identified by comparison of mass spectra and Kovats retention indices (RI) with an in-house library and the NIST17 database. The RIs were calculated using C_7 to C_{30} n-alkane standards diluted in hexane [28]. For GC-MS analysis, peaks were integrated using Agilent's Mass Hunter Unknowns Analysis 8.0 with a signal-to-noise threshold of 3, using Total Ion Chromatogram (TIC) Analysis for peak detection mode. Compounds with an area below 0.05% were excluded, and a minimum spectral match factor of 80 was applied. GC-FID peak integration was performed using Agilent CDS OpenLab ChemStation with the same signal-to-noise threshold. Quantification of the 4 major compounds in lavender extracts (linalool, linalyl acetate, lavandulol and lavandulyl acetate) was carried out according to the International Organization of the Flavor Industry (IOFI) recommended practice [29] using relative response factors (RRF) and 1-undecanol (100 μ g) as internal standard (ISTD), as described previously [30]. Briefly, the predicted RRF formula is as follows:

$$RRF_A = 10^3 (MW_A / MW_{ISTD}) \left(\frac{-61.3 + 88n_C + 18.7n_H - 41.3n_O}{+6.4n_N + 64.0n_S + 127n_{Benz}} \right)^{-1}$$

where A is the compound of interest, MW_A and MW_{ISTD} are molecular masses of A and ISTD, (undecanol) respectively, n_C , n_H , n_O , n_N and n_S are the number of carbon, hydrogen, oxygen, nitrogen and silicon atoms, respectively, while n_{Benz} is the number of benzene rings. The details of the calculations can be found in Supplementary Info.

Principal component analysis (PCA) was performed using R Statistical Software (v4.1.2; R Core Team 2021). Analysis of variance (ANOVA) was used to determine the difference in the extraction based on the composition of volatile compounds detected in samples at 95% confidence interval. Differences are significant if the p -value is less than or equal to 0.05. Tukey's Honest Significant Difference post-hoc test was used to determine significant differences for each compound. Both 2-way ANOVA

and Tukey's test were performed using Microsoft Excel 365 (2021).

3 Results and Discussion

3.1 Authentication

To verify the authenticity of the dried *L. angustifolia* sample, the in-house steam-distilled oil (S1) was analyzed alongside two commercial lavender EOs (L1, L2) and two lavandin oils (LN1, LN2). International specifications for lavender and lavandin oils are defined by ISO 3515:2002 (*L. angustifolia*) [31] and ISO 8902:2009 (*L. intermedia*) [32], both of which are based on steam-distilled oils from flowering tops. These standards specify acceptable ranges for key compounds, including linalool (20–45 % in lavender; 24–35 % in lavandin), linalyl acetate (25–47 % vs. 28–38 %), camphor (0–1.2 % vs. 6–8 %), and eucalyptol (0.5–3 % vs. 4–7 %) as shown in Table 1. Accordingly, authenticity was assessed only against steam-distilled commercial oils, as other extraction methods (e.g., SDE, SAFE) are not covered by ISO standards and may produce different compositional profiles. This comparison confirmed that the dried flowers used for in-house extraction were true lavender (*L. angustifolia*) rather than lavandin (*L. intermedia*), based on diagnostic markers such as camphor and eucalyptol contents. It also benchmarked S1 against commercial lavender oils that represent industry standards, differentiated lavender from lavandin oils often interchanged in the marketplace, and provided an industry-relevant context for evaluating how extraction methods influence aroma quality. Notably, S1 exhibited a volatile profile closely resembling that of commercial lavender EO (L2), supporting its classification as true lavender (*L. angustifolia*). Moreover, as shown in Figure 1, the first two principal components explained 75.79 % of the total variance (PC1 = 45.37 %, PC2 = 30.42 %). Lavender oils (commercial samples L1, L2) and the in-house steam-distilled oil (S1) clustered separately from lavandin oils (LN1, LN2). S1 grouped closely with L2, confirming its classification as true lavender (*L. angustifolia*). Loadings of key compounds (red arrows) indicated that linalool, linalyl acetate, camphor, and eucalyptol were the main contributors to the separation between lavender and lavandin profiles. Differences between the two commercial lavender oils (L1 and L2) may be attributed to geographical origin. Although the specific sources of the oils analyzed here were not known, previous work by Bialon *et al.*, [8]

reported that lavender oils from Australia, Italy, and Iran contain up to 1.4 % α -pinene, whereas this compound is absent in oils from China and Greece.

Similarly, lavandulyl acetate and lavandulol are absent in lavender oils from Bulgaria but are present in those from India and China [8]

Table 1: Volatile components abundance (% v/v) for *L. angustifolia* and *L. intermedia* EO as defined by ISO 3515:2002 and ISO 8902:2009.

Compound	ISO 3515:2002 <i>L. angustifolia</i>	L1	L2	S1	ISO 8902:2009 <i>L. intermedia</i>	LN1	LN2
myrcene	n.d	0.23	0.12	0.06	n.d	-	0.08
D-limonene	0.3–1	0.09	-	0.06	0.5–1.5	0.12	0.16
β -ocimene	1–10	-	0.31	0.86	0.5–1.5	-	0.34
eucalyptol	0.5–3	0.73	0.29	0.25	4–7	5.15	4.26
linalool	20–45	45.45	41.35	37.44	24–35	42.66	44.55
(-)-camphor	0–1.2	0.14	0.06	0.07	6–8	6.25	5.04
lavandulol	0–3	-	0.12	0.11	0.2–0.8	-	-
borneol	n.d	1.61	0.19	0.27	n.d	1.59	1.41
terpinen-4-ol	0–8	1.23	3.97	5.64	1.5–5	0.46	0.64
α -terpineol	0–2	0.19	0.81	0.25	n.d	0.42	0.49
linalyl acetate	25–47	44.18	46.92	49.26	28–38	41.55	41.54
lavandulyl acetate	0–8	0.26	2.49	1.93	1.5–3	0.45	0.45

ISO specifications: *L. angustifolia* (ISO 3515:2002) [31] and *L. intermedia* (ISO 8902:2009) [32].

Sample codes: S1, in-house steam-distilled lavender oil; L1 and L2, commercial lavender essential oils; LN1 and LN2, commercial lavandin EO.

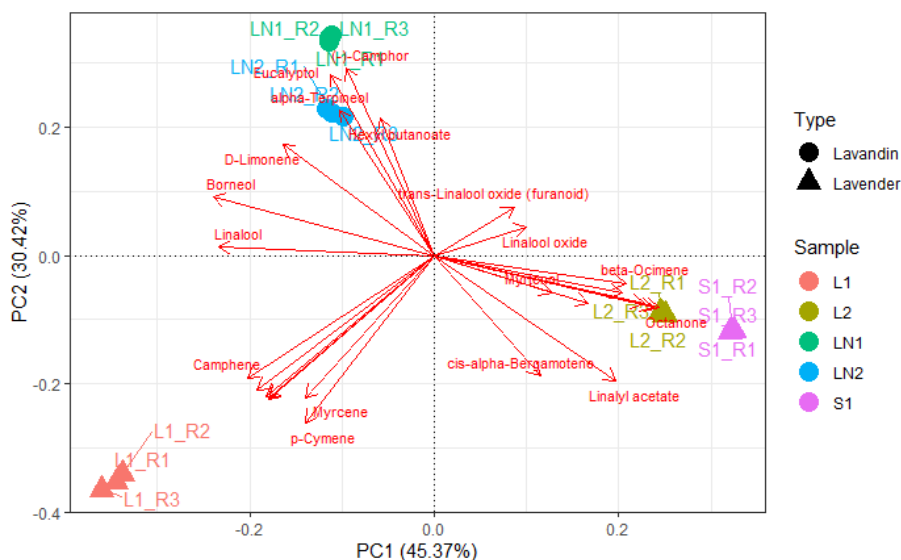


Figure 1: Principal component analysis (PCA) of volatile compounds in an in-house steam distillate, lavender (S1, L1, L2) and lavandin (LN1, LN2) EO. Sample codes: S1, in-house steam-distilled lavender oil; L1 and L2, commercial lavender EO; LN1 and LN2, commercial lavandin EO.

3.2 Comparison of extraction techniques

The dried *L. angustifolia* sample from section 3.1 was extracted via SD (S1), SDE (S2) and SAFE (S3). Table 2 compares the experimental parameters for the different extraction techniques. In terms of operational considerations, SD is generally the most cost-effective technique, SDE requires additional solvent handling and simultaneous heating of two phases, and SAFE

involves the highest methodological complexity due to solvent consumption and high-vacuum operation. In addition to distillation-based extractions, HS-SPME analysis was performed to profile the headspace volatiles of dried lavender flowers. This approach confirmed the dominance of linalool and linalyl acetate as key compounds, consistent with the distilled oils, while also detecting several minor and thermally labile constituents such as α -terpinene, allo-

ocimene, geranyl acetate, and cedr-8-ene that were less prominent or absent in the steam-distilled samples. Notably, SPME captured a higher proportion of sesquiterpenes (~8 %) compared to distilled oils (<1 %), highlighting the influence of the extraction method on compound selectivity.

Table 2: Comparison of extraction methods applied in this work.

Parameter	SD (S1)	SDE (S2)	SAFE (S3)
Amount of sample (g)	50	25	15
Moisture content (%)	11.4	11.4	11.4
Dry weight (g)	44.3	22.2	10.1
Temperature (°C)	100	100 (water) 50 (solvent)	30
Extraction time (h)	2	1	3
Final volume of extract obtained (mL)	1.4	1	1

These findings support the complementary role of SPME in reflecting aroma-active compounds

relevant to headspace perception, whereas distillation remains the benchmark for ISO-compliant EO. More detailed SPME results are provided in the Supplementary Information.

Figure 2 shows the TIC of the in-house extracted samples (S1–S3), clearly demonstrating the number of detected peaks. The peak numbers in the chromatograms are referenced with the results of the GC-MS analysis in Table 3. In total, 30 peaks were identified based on mass spectra and retention indices (RI) matches to NIST17 database, while 17 peaks remained unidentified. SAFE (S3) yielded the greatest number of peaks (29, accounting for 92 % of the total peak area), compared with 19 in SD (S1) and 17 in SDE (S2), both > 99 % identified. Two-way ANOVA confirmed significant differences in aroma profiles across methods ($p < 0.05$), with S3 differing from S1 and S2 for most volatiles (Table 3), likely due to lower extraction temperatures (Table 2). Only α -pinene, butyl butanoate, and octanone did not differ significantly.

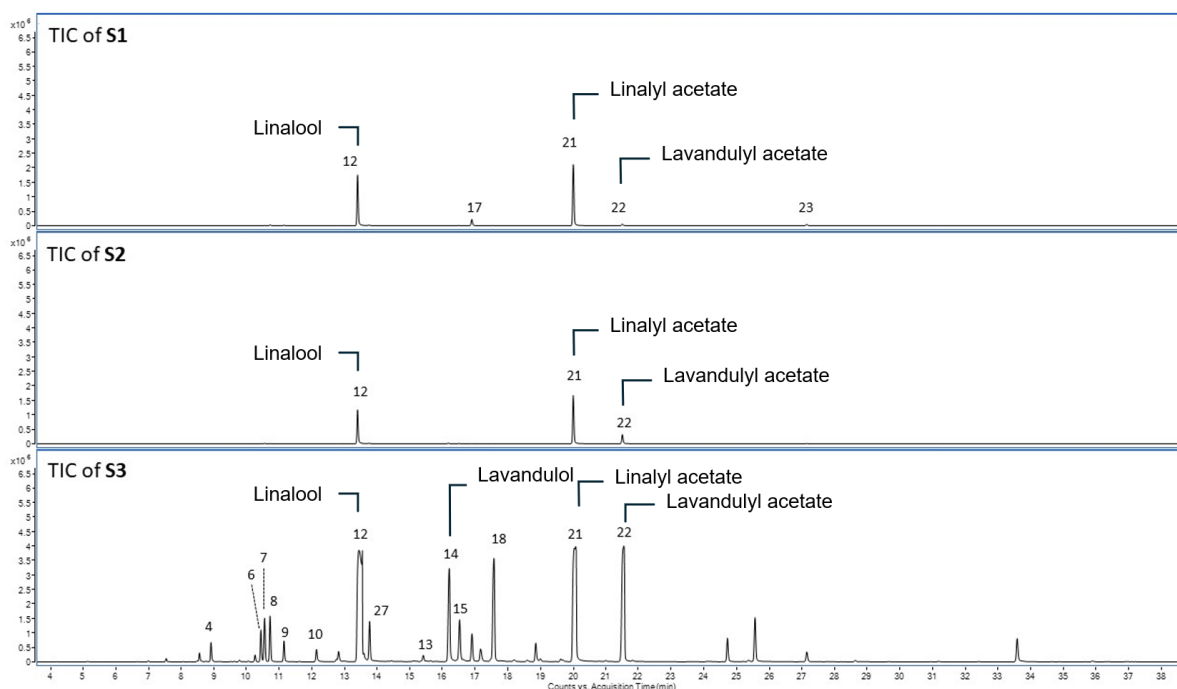


Figure 2: Representative total ion chromatograms of lavender extracts S1 – S3. Peak numbers represent the putative identifications of the compounds as numbered in Table 3.

Table 3: Volatile compounds in lavender oils and extracts (average relative area % $\pm$ standard deviation).

S/N	Compound name	RI _{cal}	RI _{ref}	Identification	Relative Area (%) ± Standard Deviation							
					S1		S2		S3			
<u>Monoterpenes / Monoterpenoids</u>												
1	α-pinene	930	932	RI, MS, Std	0.08	±	0.00		0.10	±	0.04	
2	camphene	947	947	RI, MS, Tent				0.06	±	0.00	0.27 ^{a,b} ± 0.08	
3	β-pinene	975	976	RI, MS, Std							0.08 ^{a,b} ± 0.02	
4	myrcene	988	990	RI, MS, Std							1.15 ^{a,b} ± 0.11	
5	p-cymene	1023	1024	RI, MS, Std	0.09	±	0.00		0.06	±	0.00	0.49 ^{a,b} ± 0.11
6	d-limonene	1028	1027	RI, MS, Std	0.06	±	0.00		0.28 ^a	±	0.01	1.83 ^{a,b} ± 0.15
7	eucalyptol	1031	1030	RI, MS, Std	0.25	±	0.01		0.53 ^a	±	0.02	2.56 ^{a,b} ± 0.06
8	β-ocimene	1035	1035	RI, MS, Std	0.86 ^b	±	0.02		0.26	±	0.02	2.61 ^{a,b} ± 0.01
9	α-ocimene	1045	1047	RI, MS, Std	0.59 ^b	±	0.01					1.27 ^{a,b} ± 0.13
10	cis-linalool oxide (furanoid)	1069	1070	RI, MS, Std								0.97 ^{a,b} ± 0.12
11	trans-linalool oxide (furanoid)	1086	1087	RI, MS, Std								0.83 ^{a,b} ± 0.09
12	linalool	1100	1100	RI, MS, Std	37.44 ^{b,c}	±	0.05		33.76 ^c	±	0.23	21.43 ± 0.62
13	(-)-camphor	1145	1143	RI, MS, Std	0.07	±	0.00		0.05	±	0.00	0.53 ^{a,b} ± 0.09
14	lavandulol	1162	1167	RI, MS, Std	0.11	±	0.00		1.07 ^a	±	0.02	7.17 ^{a,b} ± 0.10
15	borneol	1169	1170	RI, MS, Tent	0.27	±	0.01		0.58 ^a	±	0.02	3.12 ^{a,b} ± 0.06
16	trans-linalool oxide (pyranoid)	1172	1169	RI, MS, Std								0.23 ^{a,b} ± 0.01
17	terpinen-4-ol	1178	1176	RI, MS, Tent	5.64	±	0.03		0.21	±	0.00	1.96 ^{a,b} ± 0.05
18	α-terpineol	1192	1190	RI, MS, Tent	0.25 ^b	±	0.01		0.19	±	0.01	8.34 ^{a,b} ± 0.16
19	bornyl formate	1225	1226	RI, MS, Tent								0.24 ^{a,b} ± 0.03
20	cuminaldehyde	1239	1240	RI, MS, Std								0.24 ^{a,b} ± 0.08
21	linalyl acetate	1248	1255	RI, MS, Std	49.26 ^c	±	0.30		50.07 ^{a,c}	±	0.07	16.35 ^{a,b} ± 0.62
22	lavandulyl acetate	1282	1291	RI, MS, Std	1.93	±	0.06		11.00 ^{a,c}	±	0.08	13.09 ± 0.55
<u>Sesquiterpenes / Sesquiterpenoids</u>												
23	cis-α-bergamotene	1414	1414	RI, MS, Tent	1.58 ^{b,c}	±	0.03		0.51	±	0.01	0.85 ^a ± 0.10
24	caryophyllene oxide	1576	1580	RI, MS, Std					0.15 ^a	±	0.00	1.93 ^{a,b} ± 0.14
<u>Alkyl/ Alkenyl esters</u>												
25	butyl butanoate	996	995	RI, MS, Tent	0.03	±	0.00					
26	hexyl acetate	1012	1013	RI, MS, Tent								0.14 ^{a,b} ± 0.03
27	1-octen-3-yl acetate	1108	1111	RI, MS, Tent	1.04	±	0.12		1.12	±	0.19	2.54 ^{a,b} ± 0.03
<u>Others</u>												
28	1-octen-3-ol	978	977	RI, MS, Std								0.6 ^{a,b} ± 0.08
29	octanone	984	984	RI, MS, Tent	0.12	±	0.00					0.08 ± 0.01
30	cryptone	1184	1184	RI, MS, Tent	0.06	±	0.00		0.05	±	0.00	1.19 ^{a,b} ± 0.08

RI_{cal}: Calculated retention indices; RI_{ref}: Reference retention indices from NIST17; MS_{tent}: tentatively identified by MS; MS_{Std}: identified by chemical standard; SD: Steam distillation; SDE: Simultaneous distillation extraction; SAFE: Solvent assisted flavour extraction; Tukey's test results – ^a: significantly different from S1; ^b: significantly different from S2; ^c: significantly different from S3.

The components of lavender extract were dominated by monoterpene alcohols and esters like linalool, linalyl acetate, lavandulol and lavandulyl acetate. Monoterpenes and monoterpene derivatives accounted for 97 % and 98 % in S1 and S2, respectively, but only accounted for 82 % in S3 (Table 3). Different extraction techniques had a large impact on their relative compositions. For example, 2 main characteristic components of lavender, linalool and linalyl acetate, exceeded 80 % in S1 and S2 but dropped to 37 % in S3, whereas lavandulol increased from <1 % (S1, S2) to >7 % in S3, and lavandulyl acetate from ~2 % (S1) to 11–13 % (S2, S3). The absolute quantification of linalool, linalyl acetate, lavandulol and lavandulyl acetate is discussed in the latter section of this paper. Furthermore, minor monoterpenes such as β -ocimene and limonene were also markedly higher in S3 (2.6 %) than in S1 (0.86 %) or S2 (0.26 %), consistent with reports of thermal oxidation during prolonged heating [33], [34]. β -pinene was detected only in trace amounts in S3 and was absent in S1 and S2, likely reflecting geographical

origin, as Bialon *et al.*, [8] previously reported the absence of β -pinene in lavender EO from China and Greece. Except for linalool and terpinen-4-ol, SAFE (S3) consistently extracted the highest levels of monoterpene alcohols, including 2.3 % eucalyptol and 8.3 % α -terpineol, compared with <1 % in S1 and S2. In contrast, S1 yielded the most terpinen-4-ol (5.6 %), likely due to longer distillation times, as observed by Zheková *et al.*, [33]. Heat-induced oxidation of limonene and linalool to α -terpineol and terpinen-4-ol has similarly been reported by Perez-Lopez *et al.*, [34]. Sesquiterpenes exceeded 1 % in S1 (1.6 %) and S3 (2.8 %) but were lower in S2 (0.7 %). Linalool oxides were detected only in S3 (0.23–0.97 %), while esters and other minor compounds accounted for 5 % in S3 compared with 0.5–2 % in S1 and S2. Linalool oxides are associated with fresh, floral, and slightly woody notes, while sesquiterpenes such as cedr-8-ene contribute subtle woody and balsamic nuances, supporting the view that SAFE provides a more representative aroma profile.

Overall, the SAFE technique (S3) yielded a broader volatile profile than distillation techniques, SD (S1) and SDE (S2), as its lower extraction temperatures preserved thermally labile monoterpenes, which could have possibly degraded or altered when exposed to water at high temperature. A greater number of minor compounds were extracted using SAFE (S3), which were otherwise absent in SD and SDE. This suggests that these minor compounds are thermally labile and do not typically survive the steam distillation process. Similar results were found in the saffron sample by Amanpour *et al.*, (2015) [35]. These researchers worked with 4 different aroma extraction methods and found the SAFE method gave a more representative aroma extract than the others for volatile analysis [35].

Additionally, the presence of eucalyptol and camphor in the extracts may reflect contributions from plant tissues other than flowers, such as leaves, which are known to contain higher levels of these compounds. Studies conducted by Hajhashemi *et al.*, [36] and Torabbeigi *et al.*, [37] reported that lavender leaves are characterized by high amounts of eucalyptol and camphor, while the flowers are characterized by linalool and linalyl acetate. These findings demonstrate a clear trade-off between extraction yield and compositional breadth. Steam distillation, which produces extracts dominated by ISO-regulated major odorants, is therefore most suitable for commercial EO production and routine quality control. In contrast, SAFE preserved a wider range of thermally labile and minor volatiles, providing a more representative aroma profile. Accordingly, the choice of extraction method should be guided by the analytical objective, with SD preferred for yield-driven applications and SAFE for comprehensive aroma characterization and sensomics studies. Although SAFE provided a more chemically diverse profile, the relative concentrations of linalool and linalyl acetate did not fall within the compositional ranges specified by ISO 3515:2002 for *L. angustifolia* EO, and the extraction yield was substantially lower than that obtained by SD. Therefore, S3 does not meet ISO criteria for commercial lavender EO, but is better suited for analytical purposes where comprehensive volatile profiling is required.

3.3 Quantification of targeted volatile compounds using GC-FID

Quantification of linalool, linalyl acetate, lavandulol, and lavandulyl acetate provided further insight into

how extraction methods influence the absolute recovery of key lavender odorants (Figure 3). These four compounds are major contributors to the characteristic aroma of lavender EO [7] and are specifically regulated in ISO 3515:2002, making their accurate quantification highly relevant for both authenticity and quality assessment. When normalized to the initial flower mass, steam distillation (S1) consistently produced the highest yields, with linalool at 7.4 mg/g, linalyl acetate at 10.1 mg/g, lavandulol at 1.0 mg/g, and lavandulyl acetate at 3.4 mg/g. SDE (S2) gave intermediate values, whereas SAFE (S3) yielded the lowest amounts of all four target compounds. These results confirm that steam distillation is the most efficient technique for maximizing the yield of ISO-defined key aroma constituents, directly supporting commercial relevance and aroma intensity. However, when considered alongside GC-MS data showing that SAFE captures a broader range of minor, thermally labile volatiles, a trade-off emerges between yield and compositional diversity. Thus, while SD best reflects industry and ISO standards, SAFE offers complementary insights into aroma authenticity and headspace perception. A direct comparison of the in-house SD sample (S1) with commercial lavender oils (L1, L2) is provided in the supplementary Information (Table A1).

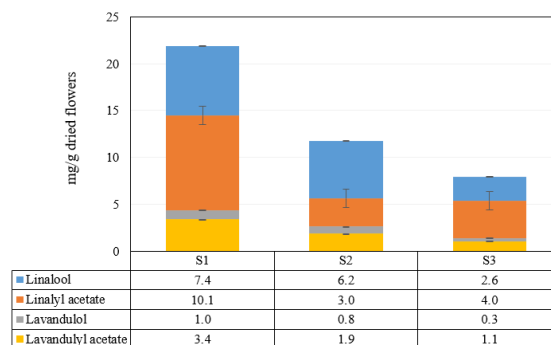


Figure 3: Quantified masses of volatile compounds (mg/g) in S1, S2 and S3 using GC-FID.

4 Conclusions

This work compared three extraction techniques—steam distillation (SD), simultaneous distillation–extraction (SDE), and solvent-assisted flavor evaporation (SAFE)—for dried *L. angustifolia* flowers. SD yielded the highest concentrations of key odorants (linalool, linalyl acetate, lavandulol, lavandulyl acetate), while SAFE produced the

broadest volatile profile by preserving thermally labile and minor compounds that contribute to aroma complexity. Two-way ANOVA confirmed significant differences among techniques, with SAFE showing the greatest variation. This variation does not indicate lower reliability but rather reflects SAFE's ability to capture a wider chemical spectrum, resulting in a more complex profile compared with the more selective SD and SDE extracts. Together, these findings highlight a trade-off: SD maximizes yield and aligns with ISO standards, whereas SAFE better represents aroma authenticity, offering complementary insights into lavender quality and sensomics relevance. From an application perspective, SD is most suitable for commercial EO production and ISO-based quality control, whereas SAFE is advantageous for sensomics, authenticity studies, and high-value flavor and fragrance applications requiring comprehensive volatile profiling.

Acknowledgments

This research was funded by Singapore Institute of Food and Biotechnology Innovation (SIFBI), Agency for Science, Technology and Research (A*STAR) for funding this research under IAF PP-grant (H17/01/a0/006).

Author Contributions

Conceptualization, N.B. and A.T.; methodology, N.B. and A.T.; experimental, N.B. and G.C.; validation, N.B. and A.T.; formal analysis, N.B.; resources, N.B., G.C., and A.T.; data curation, N.B.; writing- original draft preparation, N.B.; writing – review and editing, N.B., G.C., P.N., and A.T.; visualization, N.B.; supervision, P.N. and A.T.; project administration, N.B.

Conflicts of Interest

The authors declare no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors utilized the AI tools to enhance the language and readability of the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online [here](#).

References

- [1] B. Salehi *et al.*, “Plants of the genus *Lavandula*: From farm to pharmacy,” *Natural Product Communications*, vol. 13, pp. 1385–1402, 2018, doi: 10.1177/1934578X1801301037.
- [2] L. Lesage-Meessen, M. Bou, J. C. Sigoillot, C. B. Faulds, and A. Lomascolo, “Essential oils and distilled straws of lavender and lavandin: A review of current use and potential application in white biotechnology,” *Applied Microbiology and Biotechnology*, vol. 99, pp. 3375–3385, 2015, doi: 10.1007/s00253-015-6511-7.
- [3] R. P. Adams and T. Yanke, “Kashmir lavender oil: A comparison of new Kashmir lavender oils with commercial lavender oils,” *Perfumer & Flavorist*, vol. 32, no. 7, pp. 40–44, 2007.
- [4] M. Karapandzova, I. Cvetkovikj, G. Stefkov, V. Stoimenov, M. Crvenov, and S. Kulevanova, “The influence of duration of the distillation of fresh and dried flowers on the essential oil composition of lavandin cultivated in Republic of Macedonia,” *Macedonian Pharmaceutical Bulletin*, vol. 58, pp. 31–38, 2012, doi: 10.33320/maced.pharm.bull.2012.58.004.
- [5] S. Singh, V. Singh, G. K. Babu, V. K. Kaul, and P. S. Ahuja, “Economics of lavender (*Lavandula officinalis* L.) in Himachal Pradesh,” *Journal of Non-Timber Forest Products*, vol. 14, no. 2, pp. 97–100, 2007, doi: 10.54207/bsmps2000-2007-JOVIG8.
- [6] J. Góra and A. Lis, “The most valuable oils – lavender oil,” *Aromaterapia PTA*, vol. 2, pp. 5–11, 1995.
- [7] J. Łyczko, K. Jałoszyński, J. Surma, J. Garcia, J. Carbonell-Barrachina, and J. Szumny, “Determination of various drying methods’ impact on odour quality of true lavender (*Lavandula angustifolia* Mill.) flowers,” *Molecules*, vol. 24, no. 16, p. 2900, 2019, doi: 10.3390/molecules24162900.
- [8] M. Białon *et al.*, “Chemical composition of two different lavender essential oils and their effect on facial skin microbiota,” *Molecules*, vol. 24, no. 18, p. 3270, 2019, doi: 10.3390/molecules24183270.
- [9] L. Peterson, “The Australian lavender industry: A review of oil production and related products,” Rural Industries Research and Development Corporation, Barton, ACT, Australia, Tech. Rep., May 2002.



- [10] Y. Lin, G. Yu, S. Zhang, G. Zhu, and F. Yi, "Comparative analysis of the differences in volatile organic components of three lavender essential oils in Ili region using sensory evaluation, GC-IMS and GC-MS techniques," *Journal of Chromatography A*, vol. 1731, p. 465197, 2024, doi: 10.1016/j.chroma.2024.465197.
- [11] X. Guo and P. Wang, "Aroma characteristics of lavender extract and essential oil from *Lavandula angustifolia* Mill.," *Molecules*, vol. 25, no. 23, p. 5541, 2020, doi: 10.3390/molecules25235541.
- [12] S. Stamova, Z. Petkova, L. Dimitrova, and E. Georgieva, "Antimicrobial activity of lavender essential oil from *Lavandula angustifolia* cultivated in northeastern Bulgaria," *Antibiotics*, vol. 14, no. 7, p. 656, 2025, doi: 10.3390/antibiotics14070656.
- [13] C. Sell, *The Chemistry of Fragrances: From Perfumer to Consumer*, 2nd ed. Cambridge, UK: Royal Society of Chemistry, 2006.
- [14] A. Wesolowska *et al.*, "Influence of distillation time on the content and composition of essential oil isolated from lavender (*Lavandula angustifolia* Mill.)," *Herba Polonica*, vol. 56, no. 4, pp. 24–36, 2010.
- [15] S. T. Likens and G. B. Nickerson, "Detection of certain hop oil constituents in brewing products," *Proceedings of the Annual Meeting – American Society of Brewing Chemists*, vol. 22, no. 1, pp. 5–13, 1964, doi: 10.1080/00960845.1964.12006730.
- [16] J. Pawliszyn, *Solid Phase Microextraction: Theory and Practice*, New York: Wiley-VCH, 1997.
- [17] W. Engel, W. Bahr, and P. Schieberle, "Solvent-assisted flavour evaporation—A new and versatile technique for the careful and direct isolation of aroma compounds from complex food matrices," *European Food Research and Technology*, vol. 209, no. 3–4, pp. 237–241, 1999, doi: 10.1007/s002170050486.
- [18] C. Da Porto, D. Decorti, and I. Kikic, "Flavour compounds of *Lavandula angustifolia* L. to use in food manufacturing: Comparison of three different extraction methods," *Food Chemistry*, vol. 112, no. 4, pp. 1072–1078, 2009, doi: 10.1016/j.foodchem.2008.07.015.
- [19] C. Weurman, P. J. Groenen, and L. J. van Gemert, "Experiments on "high-vacuum transfer" in food odour research," *Nahrung*, vol. 14, no. 7, pp. 607–616, 1970, doi: 10.1002/food.19700140709.
- [20] P. Schieberle, "Quantification of important roast-smelling odorants in popcorn by stable isotope dilution assay and model studies on flavor formation during popping," *Journal of Agricultural and Food Chemistry*, vol. 43, no. 9, pp. 2442–2448, 1995, doi: 10.1021/jf00057a037.
- [21] M. Majcher and H. H. Jeleń, "Comparison of suitability of SPME, SAFE and SDE methods for isolation of flavor compounds from extruded potato snacks," *Journal of Food Composition and Analysis*, vol. 22, no. 7–8, pp. 606–612, 2009, doi: 10.1016/j.jfca.2008.11.006.
- [22] J. M. Blakeway, M. L. Frey, S. Lacroix, and M. S. Salerno, "Chemical reactions in perfume ageing," *International Journal of Cosmetic Science*, vol. 9, no. 5, pp. 203–214, 1987, doi: 10.1111/j.1467-2494.1987.tb00475.x.
- [23] D. Báez, D. Morales, and J. A. Pino, "Volatiles from *Michelia champaca* flower: Comparative analysis by simultaneous distillation-extraction and solid phase microextraction," *Natural Product Communications*, vol. 7, no. 5, pp. 659–660, 2012, doi: 10.1177/1934578X1200700529.
- [24] X. Cai *et al.*, "Analysis of aroma-active compounds in three sweet osmanthus (*Osmanthus fragrans*) cultivars by GC-olfactometry and GC-MS," *Journal of Zhejiang University Science B*, vol. 15, no. 7, pp. 638–648, 2014, doi: 10.1631/jzus.B1400058.
- [25] X. Yang, F. Yang, Y. Liu, J. Li, and H.-L. Song, "Identification of key off-flavor compounds in thermally treated watermelon juice via gas chromatography-olfactometry-mass spectrometry, aroma recombination and omission experiments," *Foods*, vol. 9, no. 2, p. 227, 2020, doi: 10.3390/foods9020227.
- [26] H. Zhang, D. Pu, B. Sun, F. Ren, Y. Zhang, and H. Chen, "Characterization and comparison of key aroma compounds in raw and dry porcini mushroom (*Boletus edulis*) by aroma extract dilution analysis, quantitation and aroma recombination experiments," *Food Chemistry*, vol. 258, pp. 260–268, 2018, doi: 10.1016/j.foodchem.2018.03.056.
- [27] N. S. Kim and D. S. Lee, "Comparison of different extraction methods for the analysis of fragrances from *Lavandula* species by gas chromatography-mass spectrometry," *Journal of Chromatography A*, vol. 982, pp. 31–47, 2002, doi: 10.1016/S0021-9673(02)01445-0.

- [28] E. Kovats, "Characterization of organic compounds by gas chromatography. Part 1. Retention indices of aliphatic halides, alcohols, aldehydes and ketones," *Helvetica Chimica Acta*, vol. 41, no. 7, pp. 1915–1932, 1958, doi: 10.1002/hlca.19580410703.
- [29] T. Cachet *et al.*, "IOFI recommended practice for the use of predicted relative-response factors for the rapid quantification of volatile flavouring compounds by GC-FID," *Flavour and Fragrance Journal*, vol. 31, no. 3, pp. 191–194, 2016, doi: 10.1002/ffj.3308.
- [30] F. Ng *et al.*, "Characterization of volatile compounds in ylang-ylang essential oils from Comoros and Madagascar by gas chromatography and principal component analysis," *Flavour and Fragrance Journal*, vol. 36, no. 2, pp. 159–166, 2021, doi: 10.1002/ffj.3625.
- [31] ISO 3515:2002 *Oil of lavender (Lavandula angustifolia Mill.)*, ISO Standard, 2002.
- [32] ISO 8902:2009 *Oil of lavandin grosso (Lavandula angustifolia Miller × Lavandula latifolia (L.f.) Medikus), French type*, ISO Standard, 2009.
- [33] G. Zhekova and N. Nedkov, "Quantitative changes in major components of lavender oil during the distillation process," *Agricultural Science and Technology*, vol. 2, no. 1, pp. 26–28, 2010.
- [34] A. J. Perez-Lopez, D. Saura, J. Lorente, and A. A. Carbonell-Barrachina, "Limonene, linalool, α -terpineol, and terpinen-4-ol as quality control parameters in mandarin juice processing," *European Food Research and Technology*, vol. 222, no. 3–4, pp. 281–285, 2006, doi: 10.1007/s00217-005-0055-5.
- [35] A. Amanpour, A. S. Sonmezdag, H. Kelebek, and S. Selli, "GC–MS–olfactometric characterization of the most aroma-active components in a representative aromatic extract from Iranian saffron (*Crocus sativus* L.)," *Food Chemistry*, vol. 182, pp. 251–256, 2015, doi: 10.1016/j.foodchem.2015.02.127.
- [36] V. Hajhashemi, A. Ghannadi, and B. Sharif, "Anti-inflammatory and analgesic properties of the leaf extracts and essential oil of *Lavandula angustifolia* Mill.," *Journal of Ethnopharmacology*, vol. 89, no. 1, pp. 67–71, 2003, doi: 10.1016/S0378-8741(03)00234-4.
- [37] M. Torabbeigi and P. A. Aberoomand Azar, "Analysis of essential oil compositions of *Lavandula angustifolia* by HS-SPME and MAHS-SPME followed by GC and GC-MS," *Acta Chromatographica*, vol. 25, no. 3, pp. 571–579, 2013, doi: 10.1556/ACHrom.25.2013.3.12.