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Direct Terpene extraction from aromatic herbs: Comparative evaluation of ultrasound-assisted stir bar sorptive extraction and ultrasound-assisted extraction by HPLC-PDA with AGREE/AGREEprep greenness assessment

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ABSTRACT

This study presents the development and comparison of two extraction methods: ultrasound- assisted stir bar sorptive extraction with liquid desorption (UA-SBSE-LD) and ultrasound-assisted extraction (UAE) for the isolation and quantification of six major terpenes and terpenoids (thymol, carvacrol, eugenol, p-cymene, γ-terpinene, and α-pinene) from culinary aromatic herbs. The analytes were analyzed by high-performance liquid chromatography with photodiode array detection (HPLC-PDA). For UA-SBSE-LD, the effects of key extraction parameters, including adsorption time, ionic strength, pH, desorption solvent composition, desorption time, and solid:liquid ratio, were investigated, while UAE was evaluated using a water–ethanol system without pH adjustment. Both techniques demonstrated excellent linearity ($R^2 \geq 0.9805$), acceptable detection limits (0.21–17 μg/mL), and satisfactory recoveries (82–120%), meeting validation requirements. Applied to thyme, oregano, rosemary, and basil, UA-SBSE-LD outperformed UAE for volatile hydrophobic compounds like γ-terpinene and α-pinene, yielding higher extraction efficiencies without organic solvent pretreatment. UA-SBSE-LD achieved up to 6.89 mg/g of α-pinene from rosemary and 4.04 mg/g of eugenol from basil. The sustainability of the methods was evaluated using AGREE and AGREEprep tools, indicating a greener profile for UAE. The direct application of UA-SBSE-LD to solid plant matrices, coupled with HPLC-PDA, represents a sensitive alternative to gas chromatography-based methods for determining terpenes in food matrices.

1. Introduction

Terpenes and terpenoids, naturally occurring bioactive compounds found in culinary aromatic plants, are critical for applications in the food industry, particularly in the study of flavor-active and bioactive components due to their antimicrobial, antioxidant, and anti-inflammatory properties (Kowalcze and Jakubowska, 2019; Pai et al., 2022; Vassiliou et al., 2023). Several compounds, including thymol, carvacrol, eugenol, p-cymene, γ-terpinene, and α-pinene, are abundant in culinary aromatic plants such as *Thymus vulgaris* (thyme), *Origanum vulgare* (oregano), *Rosmarinus officinalis* (rosemary), and *Ocimum basilicum* (basil), and are widely recognized for their biological properties

List of abbreviations: UAE, Ultrasound-assisted extraction; GC, Gas chromatography; HPLC, high-performance liquid chromatography; PDA, Photodiode array; SPME, Solid-phase microextraction; LPME, Liquid-phase microextraction; SBSE, Stir bar sorptive extraction; PDMS, polydimethylsiloxane; LD, liquid desorption; LOD, limit of detection; LOQ, limit of quantification; RSD, relative standard deviation; GAC, green analytical chemistry; GSP, green sample preparation.

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(Chuang et al., 2018; Fachini-Queiroz et al., 2012; Khalil et al., 2017; Posgay et al., 2022; Rathod et al., 2021). Although these compounds are typically obtained through the distillation of essential oils prior to analysis, their direct extraction and quantification from solid plant material remain challenging. Their volatility, hydrophobic nature, and the complexity of plant matrices complicate both extraction efficiency and accurate measurement, necessitating the use of advanced analytical methods that strike a balance between efficiency, sensitivity, and environmental sustainability. Traditional extraction techniques, such as Soxhlet extraction, maceration, and hydrodistillation, are widely used but suffer from significant drawbacks, including high solvent consumption, prolonged extraction times, and loss of volatile compounds (Palmieri et al., 2020; Roohinejad et al., 2017; Tsakni et al., 2023). Consequently, there is growing interest in miniaturized extraction techniques that provide higher efficiency while reducing sample handling and solvent use. Among these, solid-phase microextraction (SPME), liquid-phase microextraction (LPME), ultrasound-assisted extraction (UAE), and stir bar sorptive extraction (SBSE) have been widely applied (Habib et al., 2024; Peng and Cao, 2021; Zhao et al., 2023). LPME provides a solvent-efficient workflow. However, its performance decreases considerably in complex solid matrices and usually requires additional pretreatment steps (Peng and Cao, 2021). SPME, in contrast, is highly effective for the analysis of volatile compounds, but the relatively small sorbent volume limits its sensitivity for trace-level analytes (de Siqueira et al., 2024). Although recent studies, such as Zhao et al., highlight its application in essential oil extraction (Zhao et al., 2023), its sensitivity and matrix compatibility remain inferior to SBSE, which uses a larger polydimethylsiloxane (PDMS) coating volume for enhanced adsorption capacity (de Siqueira et al., 2024; Zhao et al., 2023). Considering these limitations, there is a necessity for efficient and accessible analytical techniques for the direct extraction and quantification of terpenes from solid plant material. This is particularly important for methods that ensure high sensitivity and robustness while reducing solvent use and sample-handling steps. SBSE, introduced by Baltussen et al. (Baltussen et al., 1999), offers high sensitivity, low solvent consumption, and the ability to handle large sample volumes, making it ideal for trace analysis (Baltussen et al., 1999; He et al., 2021). Current applications of SBSE are primarily limited to liquid matrices, including wine and environmental water (He et al., 2021; Prieto et al., 2010), and its application to solid plant matrices, particularly without organic solvent pre-treatment, remains underexplored. Recent advancements, such as those by Song et al., demonstrate the potential of SBSE for heavy metal analysis in food. However, its application for terpene extraction from solids, coupled with HPLC-PDA, is still novel (Song et al., 2023).

In parallel, the UAE serves as a widespread technique in green extraction. This process improves solvent penetration via cavitation, thereby decreasing extraction time and energy usage (Tekin et al., 2015; Vo et al., 2024). While the UAE is well-established, its evaluation for specific terpenes using green solvents like water-ethanol mixtures offers new insights, particularly for polar compounds (Kumar et al., 2021; Munekata et al., 2020). However, its efficacy for non-polar, volatile terpenes like γ-terpinene and α-pinene is limited, necessitating a comparative evaluation with SBSE.

Gas chromatography (GC) is the standard analytical method for terpene analysis due to its sensitivity towards volatile compounds. However, its applicability becomes more limited when dealing with aqueous or semi-aqueous plant extracts. GC requires additional preparation steps, increasing solvent consumption and prolonging the overall analysis time (Singh et al., 2024). Furthermore, because of their low boiling points and thermal lability, GC-based analysis may be associated with partial losses during injection or volatilization steps, potentially influencing their quantitative assessment in complex plant matrices (Li, 2023). High-performance liquid chromatography (HPLC), on the other hand, provides a complementary approach with simpler workflows and compatibility with a range of matrices, offering potential advantages for high-throughput or green analytical strategies, making it a promising alternative for terpene quantification (Singh et al., 2024).

This study aims to develop and compare two extraction techniques: UA-SBSE coupled with liquid desorption (UA-SBSE-LD) and UAE, for the direct extraction of thymol, carvacrol, eugenol, p-cymene, γ-terpinene, and α-pinene from culinary aromatic herbs, followed by HPLC-PDA analysis. This work addresses a critical gap in microextraction applications by directly applying SBSE to solid plant matrices without solvent pre-treatment. Importantly, the methodology skips conventional essential oil distillation, enabling the evaluation of both methods in simplified, solvent-efficient conditions. The comparative analysis of SBSE and UAE highlights their strengths for polar and non-polar terpenes, with UAE also offering a greener sample preparation alternative. Together, these methods provide a robust and efficient analytical approach for the determination of terpene flavor compounds in food matrices.

2. Materials and methods

2.1. Materials

High-purity standard compounds were used: thymol (≥99%, Scharlab, Spain), carvacrol (98%, Aldrich, UK), eugenol (99%), p-cymene (+99%), γ-terpinene (97%), and α-pinene (97%, stabilized with α-tocopherol) (Thermo Scientific, China). Anisole (Sigma-Aldrich) served as the internal standard. Acetonitrile (HPLC grade, Sigma-Aldrich), ethanol (96%), methanol, chloroform, and NaCl (VWR). Sodium hydroxide pellets and hydrochloric acid (37%, Sigma-Aldrich) were used. Ultra-pure water was applied throughout. Commercial polydimethylsiloxane (PDMS) stir bars (20 mm × 1 mm, Twister™, Gerstel GmbH, Germany) were used for SBSE.

2.2. Sample preparation

Dried leaves of thyme (*Thymus vulgaris*), oregano (*Origanum vulgare*), rosemary (*Rosmarinus officinalis*), and basil (*Ocimum basilicum*) were purchased commercially from Fitodry®, Hungary, and stored in opaque containers at 4°C. Samples were ground using a Retsch MM200 ball mill (Germany) at 25 Hz for 5 min. The particle size (~0.5 mm) was verified by sieve analysis. To assess variability, moisture content was determined (5.2–7.8% w/w). All analytical results were expressed on a dry weight basis. Ground samples were stored at 4°C in airtight containers.

A standard mixture solution (1 mg/mL) of six compounds (thymol, carvacrol, eugenol, p-cymene, γ-terpinene, α-pinene) was prepared by dissolving ~10 mg of each in acetonitrile (ACN) in a 10 mL volumetric flask, stored at 4°C, and further diluted with ultra-pure water to the required concentration for experiments.

2.3. Selection of SBSE extraction parameters

Several sets of experiments were conducted to select the extraction parameters, such as adsorption time, ionic strength, pH, desorption solvent, desorption time, desorption volume, and solid:liquid ratio, which were investigated systematically. The influence of each parameter on the recovery of target analytes was evaluated under controlled conditions, and values providing the best extraction performance were selected. All experiments were performed in triplicate using a 30 μg/mL standard mixture.

2.4. Ultrasound assisted stir bar sorptive extraction

Ground plant material (0.05 g) was mixed with 0.5 mL anisole (10 mg/mL, internal standard) and 9.5 mL ultra-pure water (20% w/v NaCl) in a 25 mL glass vial, achieving a 1:200 solid:liquid ratio. The mixture was ultrasonicated (NEY Ultrasonik 3 QT HEAT, USA, 50 Hz) for 5 min at 25°C. A PDMS stir bar was stirred at 200 rpm for 20 min. Post-extraction, the stir bar was rinsed with ultra-pure water, dried with

tissue paper, and desorbed in 500 μL ACN:chloroform (40:60) for 30 min at 200 rpm. Stir bars were cleaned in 0.5 mL ACN:methanol (50:50) for 5 min and reused up to 50 times with appropriate reconditioning, without significant loss of performance or visible degradation. After multiple uses of the stir bar, a blank desorption run was performed to check for memory effects, and no carry-over was observed. Blanks analyzed by HPLC-PDA confirmed no carryover (< 0.1 μg/mL analyte detected).

2.5. Selection of UAE extraction parameters

UAE parameters, including solvent compositions (varying ratios of water and ethanol), extraction times, and pH levels, were examined to achieve the best extraction efficiency. Thyme samples were used, with experiments in triplicate.

2.6. Ultrasound-assisted extraction

Ground plant material (0.05 g) was mixed with 10 mL of 60:40 water:ethanol in a 10 mL volumetric flask (1:200 solid:liquid ratio), vortexed for 1 min, and ultrasonicated at 50 Hz, 25°C for 30 min. Extracts were filtered through filter paper and a 0.45 μm PTFE filter.

2.7. HPLC analysis

An ECOM Quarternary HPLC system (ECS05) with a photodiode array (PDA) detector and autosampler was used with an RP-C18 column (250 mm × 4.6 mm, 3 μm, Phenomenex, USA). Gradient elution started at 50:50 acetonitrile:water for 5 min, increased to 100% acetonitrile over 10 min, and returned to 50:50 over 5 min (flow rate: 0.8 mL/min, injection volume: 5 μL, column temperature: 25°C). UV detection was performed at 217 nm for p-cymene, γ-terpinene, and α-pinene, and at 274 nm for thymol, carvacrol, and eugenol. Compounds were identified by comparing their retention times with those of standards using Clarity software (DataApex, Czech Republic). Peak purity was checked by comparing PDA spectra at the apex and along the peak profile, and no spectral mismatch was observed.

2.8. UA-SBSE and UAE method validation

Both methods were validated for linearity, limits of detection (LOD), limits of quantification (LOQ), precision, and accuracy. Calibration curves for thymol, carvacrol, eugenol, p-cymene, γ-terpinene, and α-pinene used peak area ratios (analyte:internal standard). LOD and LOQ were determined for each compound based on signal-to-noise ratios of 3 and 10, respectively. Intra-day precision (n = 6, thyme samples) and inter-day precision (n = 6, two days) were expressed as relative standard deviations (RSDs). The accuracy of UA-SBSE was assessed by spiking three concentration levels per compound (low, medium, high) in triplicate.

2.9. Statistical analysis

Experiments were conducted in triplicate, with data analyzed using GraphPad Prism 9.0, and results were presented as mean ± SD. The Shapiro–Wilk test was performed to confirm the normality of data distribution with $p > 0.05$. One-way ANOVA with Tukey's post-hoc test was used to assess parameter effects, and Student's *t*-test was applied for pairwise comparisons of each compound within the same plant matrix between the two developed extraction methods (UA-SBSE-LD and UAE). Statistical significance was considered at $p < 0.05$. The charts were generated using GraphPad Prism 9.0.

3. Results and discussion

This study developed and evaluated two extraction techniques (UA-SBSE-LD and UAE) coupled with HPLC-PDA for the determination of terpenes and terpenoids in culinary aromatic herbs (thyme, oregano, rosemary, and basil). The target analytes included thymol, carvacrol, eugenol, p-cymene, γ-terpinene, and α-pinene, which are key volatile compounds contributing to the herbs' aroma, flavour and bioactivity. The following sections detail the selection and evaluation of extraction parameters, assess method performance, and compare their effectiveness in real sample analysis, supported by comparisons to existing literature.

3.1. Selection of UA-SBSE extraction parameters

To determine the target compounds (thymol, carvacrol, eugenol, p-cymene, γ-terpinene, and α-pinene), various parameters were systematically evaluated using a standard solution (30 μg/mL of each analyte). Ultrasound treatment was applied as a fixed pre-treatment step (5 min) prior to extraction and was not included as a variable in the optimization. The factors examined included adsorption time (10, 20, 30, 40, 60 min), ionic strength (0%, 10%, 20%, 30% NaCl w/v), pH (4, 6, 8), desorption solvent (ACN, chloroform, methanol, ethanol, and ACN: chloroform mixtures at 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80), desorption time (5, 10, 15, 20, 30, 40 min), desorption volume (200, 300, 500, 1000 μL), and sample amount (solid:liquid ratios of 1:25, 1:50, 1:100, 1:200).

3.1.1. Selection of optimal desorption solvent

The desorption solvent must maximize analyte recovery from the stir bar while ensuring compatibility with the PDMS coating and HPLC system. Solvents tested included ACN, chloroform, methanol, ethanol, and ACN:chloroform mixtures (80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80). Pure ACN and methanol, commonly used in SBSE due to their polarity (Song et al., 2023), were less effective for the hydrophobic terpenes in this study. Chloroform alone provided high recovery for carvacrol and eugenol, but resulted in peak broadening and co-elution of thymol and carvacrol, affecting quantification accuracy. Despite its lower alignment with green chemistry principles, chloroform demonstrated consistent chromatographic stability across replicates, justifying its inclusion during development.

The ACN:chloroform (40:60) mixture emerged as the optimal solvent, providing the highest extraction efficiencies for thymol, p-cymene, γ-terpinene, and α-pinene ($p < 0.001$) (Fig. 1a). This ratio combines chloroform's affinity for non-polar compounds with ACN's ability to enhance chromatographic resolution, avoiding the peak overlap seen with pure chloroform. The 40:60 ratio ensures sufficient polarity for moderately polar analytes like thymol while preserving HPLC compatibility, making it suitable for this study.

3.1.2. The effect of adsorption time

Adsorption time determines the extent of analyte partitioning into the PDMS phase. Testing durations from 10 to 60 min (Fig. 1b) revealed that recovery of thymol, carvacrol, and eugenol increased significantly from 10 to 20 min ($p < 0.01$), plateauing thereafter and indicating equilibrium at 20 min. The recovery of p-cymene, γ-terpinene, and α-pinene was consistent across replicates, with no significant differences detected, likely reflecting their efficient partitioning behavior resulting from high hydrophobicity.

In SBSE, equilibrium reflects a balance where analyte uptake by PDMS matches its release back into the sample (Baltussen et al., 1999). Prolonged extraction potentially leads to re-equilibration or degradation, particularly for volatile terpenes. Thus, 20 min was selected as the optimal duration, offering efficiency and consistency across all compounds while minimizing analysis time.

3.1.3. pH and ionic strength effects

Sample pH can alter analyte ionization, affecting PDMS partitioning. Still, the tested pH values (4, 6, 8) showed no significant impact on

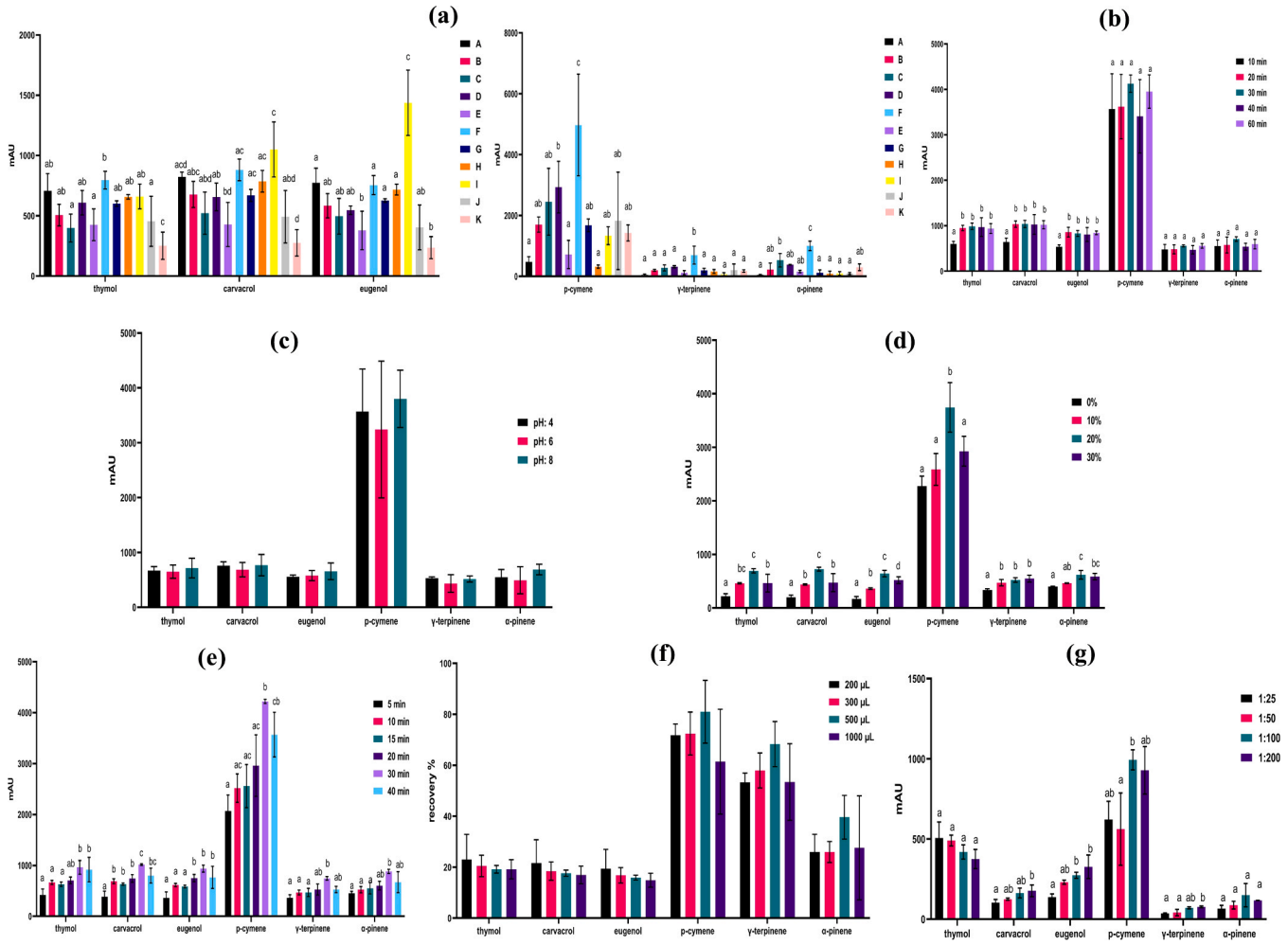

Fig. 1. Effects of (a) desorption solvent composition, (b) adsorption time, (c) pH, (d) ionic strength, (e) desorption time, (f) desorption solvent volume, and (g) sample amount (solid-to-liquid ratio) on the recovery of analytes using UA- SBSE-HPLC-PDA. Data are presented as mean ± SD (n = 3). Different letters indicate statistically significant differences (one-way ANOVA, Tukey's test). No significant differences were observed for pH (c) and desorption solvent volume (f) ($p > 0.05$). More stringent significance levels were applied for (a) ($p < 0.001$) and (b,d) ($p < 0.01$). Solvent compositions are provided in table (S1).

recovery ($p > 0.05$) (Figure S1, Fig. 1c), as thymol (pK_a 10.6), carvacrol (pK_a 10.4), and eugenol (pK_a 10.2) remain non-ionized below pH = 8. This eliminates the need for pH adjustment, simplifies the method and protects the PDMS coating from acidic or basic degradation, a concern raised by Prieto et al. (Prieto et al., 2010).

Ionic strength has a significant influence on the distribution of target compounds in solution. To evaluate this, NaCl was added to the aqueous extraction solution at concentrations of 0–30% (w/v). As shown in Fig. 1d, the extraction efficiency of thymol, carvacrol, eugenol, and p-cymene increased significantly ($p < 0.01$) with NaCl concentrations rising from 0% to 20%, indicating an optimal ionic strength at 20% NaCl. However, further increasing NaCl to 30% reduced the extraction efficiency. Conversely, γ-terpinene extraction efficiency showed slight but non-significant improvement at higher salt levels. Similarly, α-pinene recovery enhanced from 10% to 20% NaCl and stabilized thereafter.

This behavior results from NaCl's dual effects during SBSE, where NaCl is commonly used to adjust ionic strength. The "salting-out" effect reduces the solubility of hydrophobic compounds, promoting their transfer to the sorbent (Habib et al., 2024). However, high NaCl concentrations increase solution viscosity, slowing molecular transfer and reducing extraction efficiency (Prieto et al., 2010; Zhong et al., 2016). Thus, 20% (w/v) NaCl was determined as the optimal ionic strength for SBSE of the target compounds.

3.1.4. *Desorption time and volume*

Desorption time ensures complete elution of the analyte from the PDMS bar. Times of 5–40 min were tested with ACN:chloroform (40:60). Equilibrium was achieved at 30 min for most compounds ($p < 0.05$, Fig. 1e), with longer times offering no benefit and risking re-adsorption or degradation. Thus, 30 min was selected.

Desorption volumes (200, 300, 500, 1000 µL) showed no significant differences in recovery ($p > 0.05$) (Fig. 1f), consistent with Brossa et al., who found 0.5–2 mL sufficient for SBSE desorption. A 500 µL volume was chosen to balance analyte concentration for HPLC injection and minimize dilution effects (Brossa et al., 2005).

3.1.5. *Sample amount (solid:liquid ratio)*

The effect of sample amount on extraction efficiency was assessed by testing solid:liquid ratios of 1:25, 1:50, 1:100, and 1:200, using a representative culinary herb (thyme). To achieve these ratios, 0.4 g, 0.2 g, 0.1 g, and 0.05 g of thyme were extracted with 9.9 mL of extraction solvent and spiked with 100 µL of a 1 mg/mL mixed standard solution of target compounds.

As shown in Fig. 1g, the maximum recoveries of carvacrol, eugenol, p-cymene, and γ-terpinene occurred at the lowest sample amount (1:200 ratio). For thymol and α-pinene, no significant differences were observed across the tested ratios. Increasing sample mass can introduce matrix-related limitations in SBSE, where competing substances reduce

selectivity and extraction efficiency by vying for sorbent binding sites (Camino-Sánchez et al., 2014). Additionally, increased plant material can elevate the viscosity of the aqueous solution, impeding analyte diffusion into the polymer coating and lowering extraction efficiency (Sereshti & Samadi, 2014). Furthermore, plant constituents may become trapped in the polymer coating's pores, competing with target analytes for adsorption. Consequently, 0.05 g (corresponding to a 1:200 solid: liquid ratio) was identified as the optimal sample amount for extracting target compounds from thyme, oregano, rosemary, and basil leaves.

Based on these findings, the optimal conditions for SBSE of thymol, carvacrol, eugenol, p-cymene, γ-terpinene, and α-pinene are: adsorption time of 20 min, 20% (w/v) NaCl in the sample solution, no pH adjustment, desorption solvent of ACN:chloroform (40:60), desorption time of 30 min, desorption solvent volume of 500 μL, and sample amount of 0.05 g (1:200 solid:liquid ratio).

3.2. UA-SBSE-HPLC-PDA performance

Table 1 summarizes the analytical performance of the UA-SBSE-HPLC-PDA method for quantifying the six target terpenes under selected conditions. The method demonstrated good linearity, with correlation coefficients (R^2) ranging from 0.9805 to 0.9963, across the following concentration ranges: 0.004–1 mg/mL for thymol, 0.004–0.5 mg/mL for carvacrol and eugenol, and 0.004–2 mg/mL for p-cymene, γ-terpinene, and α-pinene. Limits of detection (LODs) ranged from 0.22 μg/mL to 17 μg/mL, while limits of quantification (LOQs) ranged from 0.75 μg/mL to 59 μg/mL, indicating high sensitivity. The enrichment factors range from 16.2 to 19.2 fold. This method's maximum enrichment factor was 20 times, assuming 10 mL of sample solution and 500 μL of desorption solution. The experimentally obtained enrichment factors were consistent with the theoretical EF value, confirming effective pre-concentration by the SBSE procedure. The relatively higher LODs observed for α-pinene and γ-terpinene compared to thymol and carvacrol are attributed mainly to their higher volatility and partial analyte loss during the liquid desorption step. This behavior is consistent with the known limitation of liquid desorption for highly volatile terpenes prior to HPLC analysis. In addition, the use of PDA detection, which is inherently less sensitive than MS-based detectors, may also contribute to the observed LOD range. Compared with a previous SBSE-HPLC-DAD study employing a specially designed porous aromatic framework/PDMS coating for hydrophobic polychlorinated biphenyls extraction (Zhang et al., 2023), the present study used a conventional PDMS coating and targeted more volatile terpene analytes, which may explain the comparatively higher LOD values observed for certain compounds.

Precision was evaluated through within-day and between-day repeatability, expressed as relative standard deviation (RSD), with values below 16.25% and 16.59%, respectively.

Recovery percentage (R%) of the target compounds in thyme, as a representative sample, was assessed using the standard spiking method at three different concentration levels to confirm the method's applicability across a broad concentration range. Recovery percentages were calculated using the formula:

$$R\% = \frac{C_{found} - C_{real}}{C_{added}} \times 100$$

where C_{found} is the concentration measured after spiking, C_{real} is the concentration of the compound in the unspiked sample, and C_{added} is the concentration of the standard added.

Recovery data are summarized in Table 1, S2. Overall, the UA-SBSE-HPLC-PDA method was effective, providing a faster and simpler extraction process than conventional techniques while maintaining acceptable sensitivity and precision. Precision (RSDs $\leq$ 16.25% intra-day, $\leq$ 16.59% inter-day) indicates reliable repeatability in complex plant matrices, with minor variability reflecting the volatile and sensitive nature of the compounds as well as inherent sample heterogeneity, which is consistent with previous studies (Silvosa et al., 2025). The observed recoveries in thyme (82–120%, Table 1) confirm the accuracy of the method and suggest good resistance to plant matrix interference, consistent with the reported efficacy of SBSE for terpenes (González-Hernández et al., 2024). These findings indicate that co-extracted matrix components did not significantly affect quantification.

Furthermore, chromatographic comparison between spiked and non-spiked samples demonstrated good selectivity of the method (Figure S3). Peak purity assessment using PDA spectral analysis confirmed spectral homogeneity across all analyte peaks with no evidence of co-eluting interferences.

Overall, SBSE shows high efficiency for hydrophobic compounds, attributed to PDMS affinity and terpene release from plant trichomes into the aqueous phase.

Table 1
Analytical performance of the SBSE-HPLC-PDA and UAE-HPLC-PDA methods.

	Compound	Linearity range mg/mL	R^2	Equation	LOD μg/mL	LOQ μg/mL	R%	Intra-day RSDs n = 6	Inter-day RSDs n = 6	EF
UA-SBSE-LD-HPLC-PDA Method	**Thymol**	0.004–1	0.996	y = 63.96x - 0.0437	0.25	0.85	90–92.5	7.27%	11.21%	18.5
	Carvacrol	0.004–0.5	0.9805	y = 66.14x + 0.0153	0.22	0.75	93.5–110	9.43%	16.45%	19.2
	Eugenol	0.004–0.5	0.981	y = 54.467x + 0.272	0.75	2.5	82–102.2	14.61%	15.65%	17.8
	p-cymene	0.004–2	0.9963	y = 5.1289x - 0.13	7.8	26	98–113.3	16.25%	16.59%	17.2
	γ-terpinene	0.004–2	0.9939	y = 1.783x - 0.1628	9	30	90–108.75	6.92%	10.81%	16.6
	α-pinene	0.004–2	0.9862	y = 0.906x - 0.0043	17	59	107.25–120	16.11%	16.47%	16.2
UAE-HPLC-PDA Method	**Thymol**	0.002–1	0.999	y = 1561.4x + 12.498	0.57	1.91	109.04	6.77%	6.00%	
	Carvacrol	0.002–1	0.999	y = 1496.2x + 10.005	0.59	2.00	110.72	6.14%	6.18%	
	Eugenol	0.002–1	0.999	y = 1618.8x + 9.1256	0.55	1.85	109.98	9.81%	9.81%	
	p-cymene	0.002–1	0.997	y = 4155.3x + 82.201	0.21	0.71	103.43	9.86%	9.87%	

3.3. Selection of UAE extraction parameters

UAE uses ultrasonic cavitation to disrupt plant tissues, releasing analytes into a solvent. Method development focused on thymol, carvacrol, eugenol, and p-cymene, excluding γ-terpinene and α-pinene due to poor recovery (50% and 34%) with green solvents (water, ethanol), attributed to their volatility and hydrophobicity, preventing complete partitioning during the extraction time.

3.3.1. Extraction solvent composition

Solvents tested included water, water:ethanol mixtures (80:20, 70:30, 60:40, 50:50), and 100% ethanol. Water alone yielded minimal recovery due to the analytes' low solubility (Fig. 2a). Thymol peaked at 60:40 water:ethanol, carvacrol and eugenol at 70:30, and p-cymene at 100% ethanol. The 60:40 mixture was selected, taking into account the solubility parameters, polarity index, and hydrogen bonding capacity of thymol, carvacrol, eugenol, and p-cymene, balancing efficiency and green chemistry principles. Likewise, Munekata et al., found that their extraction method using a 50:50 ethanol-water mixture significantly enhanced the recovery of phenolic compounds from thyme and rosemary (Munekata et al., 2020). These results suggest a relationship between solvent composition and extraction efficiency, in agreement with various reports.

3.3.2. Extraction time and pH

The influence of extraction time on the extraction efficiency of four target flavor compounds was evaluated over durations of 0.5, 1, 1.5, 2, 3, and 24 h, with results shown in Fig. 2b. No significant differences were observed in the extracted concentrations of thymol, carvacrol, and eugenol across the tested time points. However, p-cymene exhibited the highest extraction efficiency at 0.5 h. Prolonged ultrasound exposure may degrade analyte integrity, reducing extraction efficiency (Kumar et al., 2021). Consequently, to optimize time and energy use, an extraction time of 0.5 h was determined to be optimal for maximizing extraction efficiency for all analytes.

The influence of pH on the extraction of four target compounds was investigated across a pH range of 2–12, as depicted in Fig. 2c. Thymol, carvacrol, and p-cymene extraction efficiency showed no significant pH dependence. In contrast, eugenol exhibited maximum extraction efficiency at pH 12. This is attributed to eugenol's weak acidic nature, with a pK_a of 10.2, allowing it to exist predominantly in its deprotonated form at pH = 12, which enhances its stability and extractability. However, UAE was conducted at an unadjusted, moderate pH to maintain environmental compatibility and avoid degradation of sensitive constituents, achieving satisfactory recovery for all analytes.

These findings indicate that optimal extraction efficiencies for thymol, carvacrol, eugenol, and p-cymene were achieved using a 60:40 water-ethanol mixture at unadjusted pH, with an extraction time of 0.5 h.

3.4. HPLC development

The mobile phase was optimized to separate six compounds: eugenol, carvacrol, thymol, p-cymene, γ-terpinene, and α-pinene. Initial isocratic elution using a 50:50 ACN-water mixture resulted in poor resolution and extended retention times. A gradient elution was developed to enhance separation efficiency, starting with a 50:50 ACN-water ratio for 5 min, then a linear increase to 100% ACN over 10 min, returning to the 50:50 ratio for 5 min. A flow rate of 0.8 mL/min and a C18 column were used. This gradient achieved baseline separation of all six compounds within 20 min, with retention times of 6.96, 9.71, 10.27, 15.55, 17.53, and 18.48 min for eugenol, carvacrol, thymol, p-cymene, γ-terpinene, and α-pinene, respectively (Figures S2, S8). Baseline separation of the critical pair carvacrol–thymol was achieved (Rs = 1.65 > 1.5).

Hajimehdipoor et al. developed an HPLC method for quantifying carvacrol and thymol in thyme essential oil using an isocratic 50:50 acetonitrile-water mobile phase, reporting retention times of 13.025 and 14.236 min for carvacrol and thymol, respectively. These findings align with our results, where carvacrol eluted before thymol (Hajimehdipoor et al., 2010). However, our gradient method significantly reduced retention times, shortening the overall analysis time.

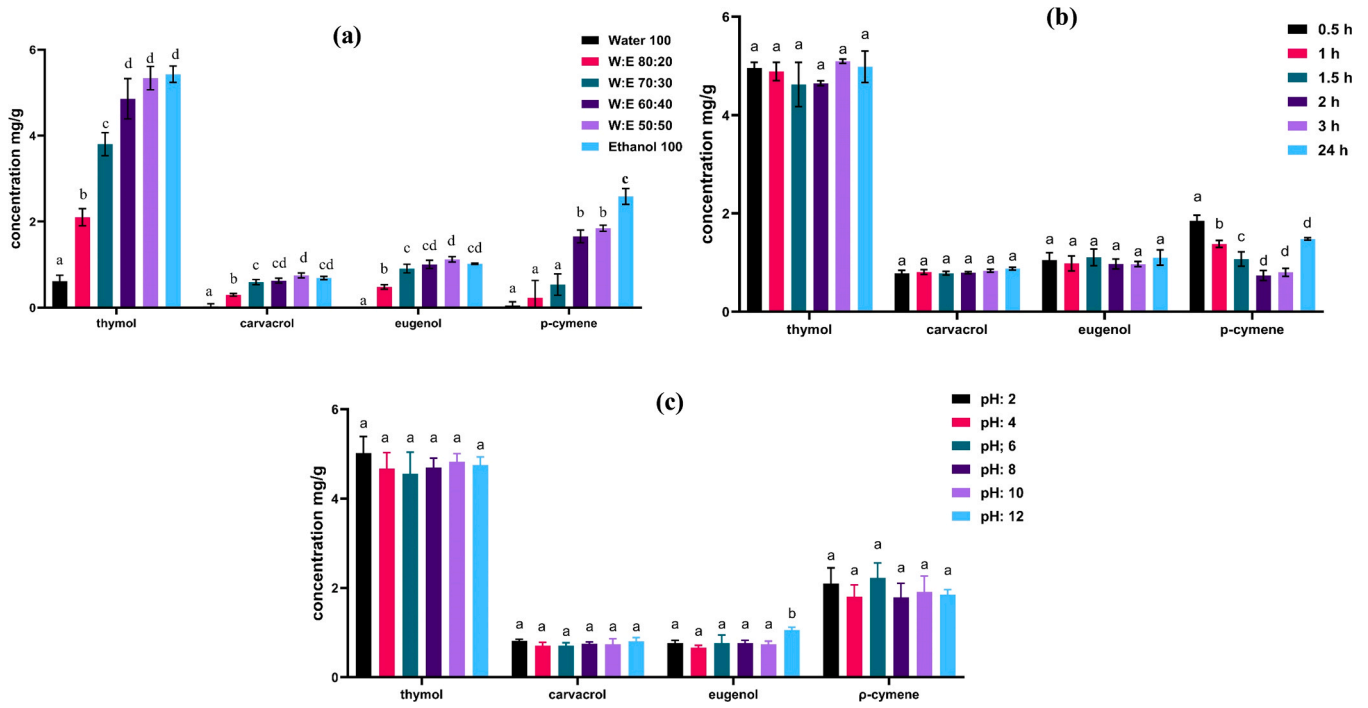

Fig. 2. Effects of (a) solvent composition, (b) extraction time, and (c) pH on the recovery of analytes using UAE-HPLC-PDA. Data are presented as mean ± SD (n = 3). W: water; E: ethanol. Bars with different letters indicate statistically significant differences according to one-way ANOVA followed by Tukey's post-hoc test ($p < 0.05$).

3.5. UAE-HPLC-PDA performance

The UAE-HPLC-PDA method was validated for the quantitative analysis of thymol, carvacrol, eugenol, and p-cymene. Extremely volatile and hydrophobic compounds (γ-terpinene and α-pinene) exhibited low recoveries with the selected green solvents (water, ethanol), limiting their inclusion in UAE validation. This choice prioritizes safety, sustainability, and applicability in industrial and food contexts, while potential future improvements, such as derivatization or natural deep eutectic solvents may improve the extraction of highly volatile and hydrophobic compounds.

LODs, (S/N = 3) ranged from 0.21 to 0.59 μg/mL, and LOQs (S/N = 10) ranged from 0.71 to 2 μg/mL. Compared to the SBSE method, UAE demonstrated higher sensitivity for p-cymene, with lower LOD and LOQ values. These values were also notably lower than those reported by Hajimehdipoor et al. for thymol and carvacrol in thyme essential oil, reporting LODs of 2.8 μg/mL for thymol and 0.6 μg/mL for carvacrol, and LOQs of 8.6 μg/mL and 1.8 μg/mL, respectively (Hajimehdipoor et al., 2010). Excellent linearity was achieved for all four compounds across a concentration range of 0.002–1 mg/mL, with correlation coefficients (R^2) between 0.997 and 0.999, as shown in Table 1.

Intra-day precision, expressed as relative standard deviation (RSD), was assessed using six replicate extractions of thyme for thymol, eugenol, and p-cymene, while oregano was selected for carvacrol because it naturally contains this compound at higher concentrations, minimizing matrix-related variability and ensuring more reliable precision assessment. The resulting intra-day RSDs were 6.14–9.86%. Inter-day precision was evaluated under the same conditions, resulting in RSDs of 6.00–9.87%.

Accuracy was assessed by spiking 100 μL of standard solution (approximately 1 mg/mL of thymol, carvacrol, eugenol, and p-cymene) into thyme samples, resulting in recoveries of 103.43–110.72%. To further evaluate the potential impact of co-extracted matrix components inherent to herbal samples, the method's selectivity was assessed through spiked recovery experiments and chromatographic analysis. The chromatograms of spiked and non-spiked samples were compared, showing no additional interfering peaks at the retention times of the target analytes (Figure S9)

Furthermore, peak purity was evaluated using PDA spectral analysis, confirming spectral homogeneity across all analyte peaks and indicating the absence of co-eluting interferences. These results demonstrate that matrix effects from co-extracted non-volatile compounds did not significantly affect the accuracy of the proposed UAE-HPLC-PDA method.

The validation results for linearity, precision, and accuracy confirm that the proposed UAE-HPLC-PDA method is reliable and reproducible for analyzing thymol, carvacrol, eugenol, and p-cymene.

A comparison of the analytical characteristics obtained in this study with previously reported GC-based methods for terpene analysis is presented in Table 2. Although GC–MS methods typically achieve lower

Table 2
Comparison of the analytical performance of the developed SBSE-HPLC-PDA and UAE-HPLC-PDA methods with previously published GC-based methods.

Compound	Method	Sample	RSD%	LOD (μg/mL)	LOQ (μg/mL)	Recovery%	Ref
Thymol	UAE (hexane)–GC–MS/MS	*Eupatorium fortunei* Turcz	2.72–2.98	0.014	0.054	106.41	(Nan et al., 2021)
	DLLME- LC-QqQ-MS^2	alcoholic beverages	10–20	0.00004–0.0009	0.00013–0.003	76–128	(Oller-Ruiz et al., 2017)
	UA-SBSE-HPLC-PDA	thyme, oregano, rosemary, and basil	7.27–11.21	0.25	0.85	90–92.5	this study
	UAE-HPLC-PDA	thyme, oregano, rosemary, and basil	6–6.77	0.57	1.91	109.04	this study
Carvacrol	HS-SPME-GC-MS/MS	bovine milk	2.8–13.6	NR	0.001	85.3 −109.6	(Armorini et al., 2016)
	hydro-distillation-HPLC-DAD	*Thymus vulgaris*	1.7–4.7	0.6	1.8	97.6 ± 1.4	(Hajimehdipoor et al., 2010)
	UA-SBSE-HPLC-PDA	thyme, oregano, rosemary, and basil	9.43–16.45	0.22	0.75	93.5–110	this study
	UAE-HPLC-PDA	thyme, oregano, rosemary, and basil	6.14–6.18	0.59	2	110.72	this study
Eugenol	Hydrodistillation–GC–MS	Hydro-distilled clove oil; commercial formulations	0.44–2.08	3.18	10.6	97.6–104.8	(Athar et al., 2013)
	SLE (methanol)-HPLC-UV	*Cinnamomum tamala* leaves &*Cinnamomum zeylanicum Breyn* stem barks	0.99–1.92	0.05	0.2	97.7–99.1	(Dighe et al., 2009)
	UA-SBSE-HPLC-PDA	thyme, oregano, rosemary, and basil	14.61–15.65	0.75	2.5	82–102.2	this study
	UAE-HPLC-PDA	thyme, oregano, rosemary, and basil	9.81	0.55	1.85	109.98	this study
p-cymene	UAE (hexane)–GC–MS/MS	*Eupatorium fortunei* Turcz	1–3.38	0.006	0.012	105.31	(Nan et al., 2021)
	UA-SBSE-HPLC-PDA	thyme, oregano, rosemary, and basil	16.25–16.59	7.8	26	98–113.3	this study
	UAE-HPLC-PDA	thyme, oregano, rosemary, and basil	9.86–9.87	0.21	0.71	103.43	this study
γ-terpinene	SHS-GC-MS	hemp-derived essential oil	2.4–10.9	0.1	0.5	95.1–111.8	(Chaiwangrach et al., 2025)
	UA-SBSE-HPLC-PDA	thyme, oregano, rosemary, and basil	6.92–10.81	9	30	90–108.75	this study
α-pinene	SHS-GC-MS	hemp-derived essential oil	2–13.6	0.025	0.1	88.7–96.5	(Chaiwangrach et al., 2025)
	UAE-CapLC-DAD	dietary supplements	11	0.25	1	98 ± 9	(Ponce-Rodríguez et al., 2021)
	Direct oil dilution–HPLC	commercial essential oils	0.38–1.97	6.92	19.29	104.42–108.65	(Porel et al., 2014)
	UA-SBSE-HPLC-PDA	thyme, oregano, rosemary, and basil	16.11–16.47	17	59	107.25–120	this study

Note: LC-QqQ-MS^2, Triple Quadrupole Mass Spectrometry with Liquid Chromatography; DLLME, Dispersive liquid-liquid microextraction; HS, headspace; SHS, static headspace; SPME, solid-phase microextraction; HS-SPME, headspace solid-phase microextraction; SLE, solid liquid extraction; GC-MS, gas chromatography–mass spectrometry; GC–MS/MS, tandem mass spectrometry; CapLC, capillary liquid chromatography; DAD, diode array detection. "NR" indicates values not reported.

detection limits, which is consistent with the higher sensitivity of mass spectrometric detection, the developed UA-SBSE-HPLC-PDA and UAE-HPLC-PDA methods showed acceptable precision and recovery, supporting their suitability as practical alternatives for the routine determination of terpenes in plant matrices.

3.6. Greenness evaluation of the developed methods

The goal of green analytical chemistry (GAC) is to develop more sustainable and environmentally friendly analytical methods. The Analytical GREEnness calculator (AGREE), one of the most current greenness evaluation tools, is among several approaches developed to date for GAC measurements (Abdelgawad et al., 2022). Analytical Greenness (AGREE) evaluation criteria consist of 12 portions corresponding to the GAC principles. These are expressed through 12 input variables, which are transformed into a common scale between 0 and 1 to generate the final result. The method is considered green if its score is higher than 0.6 (Pena-Pereira et al., 2020). Along with being quantitative, it also provides a qualitative and graphical illustration of the method's advantages and disadvantages (Mahdavi et al., 2024). The greenness of sample preparation processes is also carefully assessed by AGREEprep in accordance with the 10 principles of green sample preparation (GSP) (Wojnowski et al., 2022).

The greenness of the two developed methods (UA-SBSE-LD-HPLC-PDA and UAE-HPLC-PDA) was assessed using the Analytical GREEnness calculator (AGREE v.0.5 beta) and AGREEprep tool, as can be seen in Fig. 3, with complete documentation of every parameter to ensure reproducibility (Tables S3-S6). AGREE evaluation indicated that neither workflow is fully green, with scores of 0.44 (UA-SBSE-LD-HPLC-PDA) and 0.45 (UAE-HPLC-PDA). This is primarily because HPLC analysis involves solvent waste, energy consumption, and operator safety issues rather than the extraction process itself (Wejnerowska and Narloch, 2023). Additionally, the SBSE-LD process relied on an ACN–chloroform desorption mixture, which, while highly effective and compatible with the chromatographic method, contributed to a less favorable environmental profile and indicates opportunities for future improvement through the development of equally effective but greener alternatives. A higher AGREEprep score (0.62 versus 0.41 for SBSE-LD) was attained by the UAE workflow, which used only water and ethanol for sample preparation, because it was easier to use, required fewer hazardous solvents, and consumed less energy. However, the environmental limitations implemented by the HPLC platform are ultimately applied by both methods. Overall, the comparison shows that UAE is the more environmentally friendly sample-preparation approach, whereas SBSE-LD is still analytically robust but could use some methodological improvements, like increasing throughput and optimizing solvent use. As a result, the combined AGREE and AGREEprep evaluations provide a clear overview of each workflow's strengths and limitations, clearly defining future development paths toward more sustainable analytical protocols.

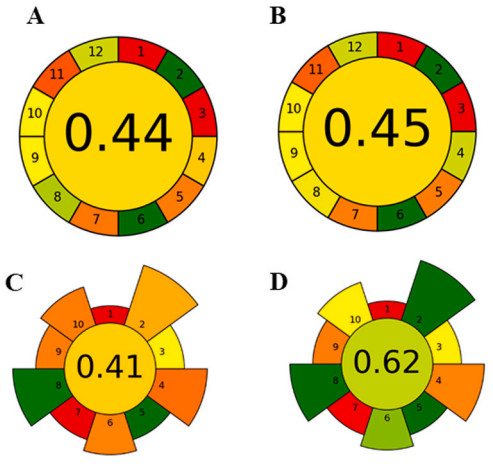

Fig. 3. Greenness and practicality evaluation of the developed methods: A) UA-SBSE-LD-HPLC-PDA by AGREE, B) UAE-HPLC-PDA by AGREE, C) UA-SBSE-LD-HPLC-PDA by AGREEprep, D) UAE-HPLC-PDA by AGREEprep.

3.7. Real sample analysis

Thyme, oregano, rosemary, and basil were analyzed using both SBSE- and UAE-based extraction methods coupled with HPLC-PDA (Figures S4–S9, S10–S13), with results summarized in Table 3. Previous studies have utilized Headspace-SPME coupled with GC to analyze volatile and semi-volatile compounds in these aromatic herbs (Díaz-Maroto et al., 2002; Mena et al., 2016; Soleimani et al., 2014). However, SBSE offers superior extraction efficiency compared to SPME, primarily due to its significantly larger extraction phase volume (50–250 times greater than SPME fibers), which minimizes competitive adsorption and enhances the recovery of volatile compounds (de Siqueira et al., 2024). This work therefore demonstrates the applicability and analytical value of a novel SBSE approach for direct terpene analysis using HPLC, an area not yet extensively explored.

In thyme samples, six target compounds were identified, with thymol being the most abundant (4.98–5.9 mg/g) regardless of the extraction method. These findings agree with earlier reports identifying thymol, p-cymene, γ-terpinene, and carvacrol as primary components of thyme oil (Dobreva et al., 2024; Wesołowska and Jadczak, 2019). Additionally, α-pinene was detected at notable levels, corroborating findings by Baj et al. (Baj et al., 2020). UAE proved more effective for extracting carvacrol and eugenol from thyme ($p < 0.05$), likely due to matrix complexity affecting SBSE performance, leading to variable extraction yields (Ruan et al., 2015). Conversely, SBSE excelled in extracting

Table 3
Terpenes and terpenoids content (mg/g) in thyme, oregano, rosemary and basil obtained using the stir bar sorptive extraction with liquid desorption coupled with high-performance liquid chromatography-photodiode array detection (UA-SBSE-LD-HPLC-PDA) and ultrasound-assisted extraction coupled with high-performance liquid chromatography-photodiode array detection (UAE-HPLC-PDA).

Plant	Method	Thymus vulgaris	Origanum vulgare	Rosmarinus officinalis	Ocimum basilicum
Thymol	SBSE	$5.9^{a} \pm 0.43$	$0.81^{a} \pm 0.02$	$0.015^{a} \pm 0.002$	$0.53^{a} \pm 0.07$
	UAE	$4.98^{b} \pm 0.3$	$0.34^{b} \pm 0.05$	$0.232^{a} \pm 0.16$	$1.55^{b} \pm 0.09$
Carvacrol	SBSE	$0.75^{a} \pm 0.07$	$3.4^{a} \pm 0.14$	ND	ND
	UAE	$1^{b} \pm 0.06$	$3.21^{a} \pm 0.19$	ND	ND
Eugenol	SBSE	$0.3^{a} \pm 0.06$	ND	ND	$4.04^{a} \pm 0.33$
	UAE	$1.06^{b} \pm 0.06$	ND	ND	$1.25^{b} \pm 0.11$
p-cymene	SBSE	$2.2^{a} \pm 0.42$	$1.95^{a} \pm 0.39$	$3.05^{a} \pm 0.62$	2.45 ± 0.3
	UAE	$1.85^{a} \pm 0.11$	$0.37^{b} \pm 0.01$	$1.32^{b} \pm 0.2$	ND
γ-terpinene	SBSE	2.43 ± 0.17	1.67 ± 0.28	2 ± 0.31	1.23 ± 0.06
	UAE	ND	ND	ND	ND
α-pinene	SBSE	1.99 ± 0.34	2.03 ± 0.31	6.89 ± 0.58	1.3 ± 0.25
	UAE	ND	ND	ND	ND

Note: Values are expressed as mean ± SD. Superscript letters (a, b) denote statistically significant differences between SBSE and UAE for the same compound in each plant ($p < 0.05$, Student's *t*-test). Abbreviations: SBSE: stir bar sorptive extraction, UAE: Ultrasound- assisted extraction, ND: not detected. Carvacrol in *R. officinalis* was present below the LOD, while all other cases of ND indicate true absence of the compound.

hydrocarbon terpenes (p-cymene, γ-terpinene, and α-pinene) due to the affinity of the apolar PDMS phase.

Comparable findings were observed in other thyme species. For instance, ethanol was reported to be an effective solvent for extracting thymol from wild thyme (*Thymus serpyllum* L.), yielding 4.92 ± 1.65 mg/g (Janiak et al., 2017) closely aligning with thymol levels in thyme obtained via UAE in this study.

A similar trend was observed in oregano, where carvacrol was the predominant compound (3.21–3.4 mg/g) across both extraction methods, consistent with previous studies (Gavarić et al., 2015; Laothaweerungsawat et al., 2020). Our findings showed higher carvacrol content than the 1999 μg/g reported by Baranauskaitė et al. (Baranauskaitė et al., 2016), possibly due to an optimized UAE protocol with a 10-minute extraction time and 96% ethanol, which may have limited extraction efficiency in earlier studies. Thymol concentrations ranged from 0.34 to 0.81 mg/g, with variations attributed to oregano chemotypes, environmental factors (including seasonal, climatic, and regional conditions), drying techniques, and genetic differences (Rodriguez-Garcia et al., 2016). The PDMS Twister bar also extracted significant levels of p-cymene (1.95 mg/g), γ-terpinene (1.67 mg/g), and α-pinene (2.03 mg/g).

In basil, SBSE revealed high eugenol content (4.04 mg/g), surpassing UAE yields and exceeding the 2.8 mg/g reported by Lenti et al. (Lenti et al., 2022) using hexane extraction. Notable levels of p-cymene, γ-terpinene, and α-pinene were also detected in basil extracts via SBSE (Table 3). For rosemary, α-pinene was the dominant terpene (6.89 mg/g), higher than the 1.47 mg/g reported by Shahina et al. (Shahina et al., 2022), with SBSE also detecting p-cymene, γ-terpinene, and trace thymol, aligning with prior studies (Mwithiga et al., 2022; Shahina et al., 2022).

Overall, Table 3 shows that UA-SBSE–HPLC-PDA provided broader compound coverage and higher extraction yields than UAE–HPLC-PDA. Although SBSE is typically applied to liquids (e.g., milk, juice, wine), this study successfully adapted UA-SBSE-HPLC-PDA for direct terpene analysis in solid herbs (thyme, oregano, basil, rosemary) without organic solvents or essential oil distillation, confirming optimal conditions for analyzing six terpenes and terpenoids.

3.8. Limitations of the study

While both UA-SBSE-LD-HPLC-PDA and UAE-HPLC-PDA demonstrated satisfactory analytical performance for terpene and terpenoid analysis in culinary herbs, some limitations should be acknowledged. The analytical efficiency of the methods varied depending on the volatility and polarity of the target analytes, particularly for highly volatile terpenes. In addition, the SBSE-LD workflow still relies on organic solvent-based desorption to ensure adequate chromatographic performance. Furthermore, the methods were evaluated using selected aromatic herbs, and additional validation in more complex plant matrices may be required.

3.9. Future perspectives

Future studies may focus on improving the recovery of highly volatile terpenes and further enhancing the environmental sustainability of the SBSE workflow through the development of greener desorption systems, novel coating materials, or miniaturized approaches. For UAE, the use of alternative green solvents, such as natural deep eutectic solvents, could improve selectivity while maintaining environmental compatibility. The integration of more sensitive detection systems, particularly MS-based detectors, as well as automation and higher-throughput platforms, may further expand the applicability of both methods in routine analysis.

4. Conclusion

This study demonstrated the applicability of UA-SBSE-LD-HPLC-PDA and UAE-HPLC-PDA for the quantitative analysis of terpenes and terpenoids in culinary aromatic herbs, including thyme, oregano, rosemary, and basil. SBSE showed superior performance for highly volatile and hydrophobic compounds due to the strong affinity of the PDMS phase, while UAE using ethanol–water mixtures was more suitable for oxygenated and relatively polar constituents and offered a greener and more scalable approach. Both methods showed acceptable precision and validation performance, with extraction efficiency mainly influenced by solvent composition, extraction time, and matrix characteristics. Overall, this comparative evaluation highlights SBSE as a sensitive laboratory-oriented tool for non-polar terpene profiling, whereas UAE provides a simpler and environmentally friendlier alternative for polar analytes. Together, the two approaches offer complementary strategies for quality control and phytochemical analysis in food, herbal, and pharmaceutical applications.

CRediT authorship contribution statement

Gréta Törős: Writing – review & editing. **Tammem Hamda:** Writing – original draft. **Tarek Alshaal:** Writing – review & editing. **Arjun Muthu:** Writing – review & editing. **Attila Kiss:** Writing – review & editing, Validation. **Áron Béni:** Writing – review & editing, Supervision. **Reina Atieh:** Writing – original draft, Validation, Methodology, Data curation, Conceptualization. **Safa Labidi:** Writing – original draft.

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

During the preparation of this work, the author(s) utilized ChatGPT to enhance language and readability. After using this tool/service, the authors reviewed and edited the content as necessary and took full responsibility for the content of the publication.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Attila Kiss reports financial support was provided by European Regional Development Fund. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jfca.2026.109273.

Data availability

Data will be made available on request.

References

Abdelgawad, M.A., Elmowafy, M., Musa, A., Al-Sanea, M.M., Nayl, A.A., Ghoneim, M.M., Ahmed, Y.M., Hassan, H.M., AboulMagd, A.M., Salem, H.F., Abdelwahab, N.S.,

2022. Development and greenness assessment of HPLC method for studying the pharmacokinetics of co-administered Metformin and Papaya extract. Molecules 27 (2). https://doi.org/10.3390/molecules27020375.
Armorini, S., Yeatts, J.E., Mullen, K.A.E., Mason, S.E., Mehmeti, E., Anderson, K.L., Washburn, S.P., Baynes, R.E., 2016. Development of a HS-SPME-GC-MS/MS method for the quantitation of thymol and carvacrol in bovine matrices and to determine residue depletion in milk and tissues. J. Agric. Food Chem. 64 (41), 7856–7865. https://doi.org/10.1021/acs.jafc.6b02899.
Athar, M.T., Tamboli, E.T., Ansari, S.H., Ahmad, S., 2013. Quantification of Eugenol in hydro-Distilled Clove Oil (*Eugenia caryophyllus*) and its marketed products by validated GC-MS method. J. Herbs Spices & Med. Plants 19 (4), 365–376. https://doi.org/10.1080/10496475.2013.807902.
Baj, T., Biernasiuk, A., Wróbel, R., Malm, A., 2020. Chemical composition and in vitro activity of *Origanum vulgare L., Satureja hortensis L., Thymus serpyllum L. and Thymus vulgaris L.* essential oils towards oral isolates of *Candida albicans* and *Candida glabrata*. Open Chem. 18 (1), 108–118. https://doi.org/10.1515/chem-2020-0011.
Baltussen, E., Sandra, P., David, F., Cramers, C., 1999. Stir bar sorptive extraction (SBSE), a novel extraction technique for aqueous samples: theory and principles. J. Micro Sep. 11 (10), 737–747. https://doi.org/10.1002/(SICI)1520-667X(1999)11:10<737::AID-MCS7>3.0.CO;2-4.
Baranauskaitė, J., Jakštas, V., Ivanauskas, L., Kopustinskienė, D.M., Drakšienė, G., Masteikova, R., Bernatonienė, J., 2016. Optimization of carvacrol, rosmarinic, oleanolic and ursolic acid extraction from oregano herbs (*Origanum onites L., Origanum vulgare spp. Hirtum* and *Origanum vulgare L.*). Nat. Prod. Res. 30 (6), 672–674. https://doi.org/10.1080/14786419.2015.1038998.
Brossa, L., Marcé, R.M., Borrull, F., Pocurull, E., 2005. Determination of endocrine disruptors in environmental water samples by stir bar sorptive extraction-liquid desorption—large volume injection-gas chromatography. Chromatographia 61 (1), 61–65. https://doi.org/10.1365/s10337-004-0447-x.
Camino-Sánchez, F.J., Rodríguez-Gómez, R., Zafra-Gómez, A., Santos-Fandila, A., Vílchez, J.L., 2014. Stir bar sorptive extraction: recent applications, limitations and future trends. Talanta 130, 388–399. https://doi.org/10.1016/j.talanta.2014.07.022.
Chaiwangrach, N., Mukda, S., Temkitthawon, P., Nuengchamnong, N., Pinmanee, S., Somboon, T., Boonnoun, P., Ingkaninan, K., 2025. Simultaneous profiling of terpenes and cannabinoids in hemp essential oils using static headspace gas chromatography–mass spectrometry for quality control and chemotype differentiation. Sci. Pharm. 93 (2), 27. https://doi.org/10.3390/scipharm93020027.
Chuang, L.-T., Tsai, T.-H., Lien, T.-J., Huang, W.-C., Liu, J.-J., Chang, H., Chang, M.-L., Tsai, P.-J., 2018. Ethanolic extract of *Origanum vulgare* suppresses Propionibacterium acnes-induced inflammatory responses in human monocyte and Mouse Ear Edema models. Molecules 23 (8). https://doi.org/10.3390/molecules23081987.
Díaz-Maroto, M.C., Pérez-Coello, M.S., Cabezudo, M.D., 2002. Headspace solid-phase microextraction analysis of volatile components of spices. Chromatographia 55 (11), 723–728. https://doi.org/10.1007/BF02491788.
Dighe, V.V., Gursale, A.A., Charegaonkar, G.A., 2009. Quantitation of Eugenol, Cinnamaldehyde and Isoeugenol from *Cinnamomum tamala* Nees and Eberm. Leaf Powder and *Cinnamomum zeylanicum Breyn* Stem Bark Powder by LC. Chromatographia 70 (11), 1759–1762. https://doi.org/10.1365/s10337-009-1382-7.
Dobreva, K., Dimov, M., Valev, T., Iliev, I., Damyanova, S., Oprea, O.B., Stoyanova, A., 2024. Chemical composition and antioxidant activities of three bulgarian garden thyme essential oils. Appl. Sci. 14 (22). https://doi.org/10.3390/app142210261.
Fachini-Queiroz, F.C., Kummer, R., Estevão-Silva, C.F., Carvalho, M.D., de, B., Cunha, J. M., Grespan, R., Bersani-Amado, C.A., Cuman, R.K.N., 2012. Effects of thymol and carvacrol, constituents of *Thymus vulgaris L.* essential oil, on the inflammatory response. Evid. -Based Complement. Altern. Med. eCAM 2012, 657026. https://doi.org/10.1155/2012/657026.
Gavarić, N., Smole Možina, S., Kladar, N., Bozin, B., 2015. Chemical profile, antioxidant and antibacterial activity of thyme and oregano essential oils, thymol and carvacrol and their possible synergism. J. Essent. Oil Bear. Plants 18. https://doi.org/10.1080/0972060X.2014.971069.
González-Hernández, R.A., Valdez-Cruz, N.A., Trujillo-Roldán, M.A., 2024. Factors that influence the extraction methods of terpenes from natural sources. Chem. Pap. 78 (5), 2783–2810. https://doi.org/10.1007/s11696-024-03339-z.
Habib, A., Noriega Landa, E., Holbrook, K.L., Chacon, A.A., Lee, W.-Y., 2024. Green analytical method for perfluorocarboxylic acids (PFCAs) in water of stir bar sorptive extraction coupled with thermal desorption–gas chromatography—mass spectroscopy. Water 16 (17). https://doi.org/10.3390/w16172543.
Hajimehdipoor, H., Shekarchi, M., Khanavi, M., Adib, N., Amri, M., 2010. A validated high-performance liquid chromatography method for the analysis of thymol and carvacrol in *Thymus vulgaris L.* volatile oil. Pharmacogn. Mag. 6 (23), 154–158. https://doi.org/10.4103/0973-1296.66927.
He, M., Wang, Y., Zhang, Q., Zang, L., Chen, B., Hu, B., 2021. Stir bar sorptive extraction and its application. J. Chromatogr. A 1637, 461810. https://doi.org/10.1016/j.chroma.2020.461810.
Janiak, M., Slavova-Kazakova, A., Kancheva, V., Ivanova, M., Tsrunchev, T., Karamać, M., 2017. Effects of γ-Irradiation of wild thyme (*Thymus serpyllum L.*) on the phenolic compounds profile of its ethanolic extract. Pol. J. Food Nutr. Sci. 67. https://doi.org/10.1515/pjfns-2017-0020.
Khalil, A.A., Rahman, U. ur, Khan, M.R., Sahar, A., Mehmood, T., Khan, M., 2017. Essential oil eugenol: sources, extraction techniques and nutraceutical perspectives. RSC Adv. 7 (52), 32669–32681. https://doi.org/10.1039/C7RA04803C.
Kowalcze, M., Jakubowska, M., 2019. Multivariate approach in voltammetric identification and simultaneous determination of eugenol, carvacrol, and thymol on boron-doped diamond electrode. Mon. F. üR. Chem. - Chem. Mon. 150 (6), 991–1002. https://doi.org/10.1007/s00706-019-02394-7.
Kumar, K., Srivastav, S., Sharanagat, V.S., 2021. Ultrasound assisted extraction (UAE) of bioactive compounds from fruit and vegetable processing by-products: a review. Ultrason. Sonochem. 70, 105325. https://doi.org/10.1016/j.ultsonch.2020.105325.
Laothaweerungsawat, N., Neimkhum, W., Anuchapreeda, S., Sirithunyalug, J., Chaiyana, W., 2020. Transdermal delivery enhancement of carvacrol from *Origanum vulgare L.* essential oil by microemulsion. Int. J. Pharm. 579, 119052. https://doi.org/10.1016/j.ijpharm.2020.119052.
Lenti, L., Rigano, D., Woo, S.L., Nartea, A., Pacetti, D., Maggi, F., Fiorini, D., 2022. A rapid procedure for the simultaneous determination of Eugenol, Linalool and fatty acid composition in basil leaves. Foods 11 (21). https://doi.org/10.3390/foods11213315.
Li, Y., 2023. Analytical methods for the analysis of volatile natural products. Nat. Prod. Rep. 40 (4), 922–956. https://doi.org/10.1039/D2NP00079B.
Mahdavi, R., Talebpour, Z., Noori, M., 2024. Application of new green evaluation tools to assess the environmental impact of analytical procedures based on solid phase microextraction techniques. Green. Anal. Chem. 10, 100144. https://doi.org/10.1016/j.greeac.2024.100144.
Mena, P., Cirlini, M., Tassotti, M., Herrlinger, K.A., Dall'Asta, C., Del Rio, D., 2016. Phytochemical profiling of flavonoids, phenolic acids, terpenoids, and volatile fraction of a rosemary (*Rosmarinus officinalis L.*) Extract. Molecules 21 (11). https://doi.org/10.3390/molecules21111576.
Munekata, P.E.S., Alcántara, C., Žugčić, T., Abdelkebir, R., Collado, M.C., García-Pérez, J.V., Jambrak, A.R., Gavahian, M., Barba, F.J., Lorenzo, J.M., 2020. Impact of ultrasound-assisted extraction and solvent composition on bioactive compounds and in vitro biological activities of thyme and rosemary. Food Res. Int. 134, 109242. https://doi.org/10.1016/j.foodres.2020.109242.
Mwithiga, G., Maina, S., Gitari, J., Muturi, P., 2022. Rosemary (*Rosmarinus officinalis L.*) growth rate, oil yield and oil quality under differing soil amendments. Heliyon 8 (4), e09277. https://doi.org/10.1016/j.heliyon.2022.e09277.
Nan, G., Zhang, L., Liu, Z., Liu, Y., Du, Y., Zhao, H., Zheng, H., Lin, R., Yang, G., Zheng, S., 2021. Quantitative determination of p-Cymene, Thymol, Neryl Acetate, and β-Caryophyllene in different growth periods and parts of *Eupatorium fortunei* Turcz. By GC-MS/MS. J. Anal. Methods Chem. 2021 (1), 2174667. https://doi.org/10.1155/2021/2174667.
Oller-Ruiz, A., Viñas, P., Campillo, N., Fenoll, J., Hernández-Córdoba, M., 2017. Triple quadrupole mass spectrometry with liquid chromatography and dispersive liquid-liquid microextraction for the determination of monoterpenes in alcoholic drinks. Food Anal. Methods 10 (11), 3615–3622. https://doi.org/10.1007/s12161-017-0937-8.
Pai, S., Hebbar, A., Selvaraj, S., 2022. A critical look at challenges and future scopes of bioactive compounds and their incorporations in the food, energy, and pharmaceutical sector. Environ. Sci. Pollut. Res. 29 (24), 35518–35541. https://doi.org/10.1007/s11356-022-19423-4.
Palmieri, S., Pellegrini, M., Ricci, A., Compagnone, D., Lo Sterzo, C., 2020. Chemical composition and antioxidant activity of thyme, hemp and coriander extracts: a comparison study of Maceration, Soxhlet, UAE and RSLDE techniques. Foods 9 (9). https://doi.org/10.3390/foods9091221.
Pena-Pereira, F., Wojnowski, W., Tobiszewski, M., 2020. AGREE—analytical GREEnness metric approach and software. Anal. Chem. 92 (14), 10076–10082. https://doi.org/10.1021/acs.analchem.0c01887.
Peng, L.-Q., Cao, J., 2021. Modern microextraction techniques for natural products. Electrophoresis 42 (3), 219–232. https://doi.org/10.1002/elps.202000248.
Ponce-Rodríguez, H.D., Verdú-Andrés, J., Campíns-Falcó, P., Herráez-Hernández, R., 2021. Capillary liquid chromatography for the determination of terpenes in botanical dietary supplements. Pharmaceuticals 14 (6). https://doi.org/10.3390/ph14060580.
Porel, A., Sanyal, Y., Kundu, A., 2014. Simultaneous HPLC determination of 22 components of essential oils; method robustness with experimental design. Indian J. Pharm. Sci. 76 (1), 19–30.
Posgay, M., Greff, B., Kapcsándi, V., Lakatos, E., 2022. Effect of *Thymus vulgaris L.* essential oil and thymol on the microbiological properties of meat and meat products: a review. Heliyon 8 (10), e10812. https://doi.org/10.1016/j.heliyon.2022.e10812.
Prieto, A., Basauri, O., Rodil, R., Usobiaga, A., Fernández, L.A., Etxebarria, N., Zuloaga, O., 2010. Stir-bar sorptive extraction: a view on method optimisation, novel applications, limitations and potential solutions. J. Chromatogr. A 1217 (16), 2642–2666. https://doi.org/10.1016/j.chroma.2009.12.051.
Rathod, N.B., Kulawik, P., Ozogul, F., Regenstein, J.M., Ozogul, Y., 2021. Biological activity of plant-based carvacrol and thymol and their impact on human health and food quality. Trends Food Sci. Technol. 116, 733–748. https://doi.org/10.1016/j.tifs.2021.08.023.
Rodriguez-Garcia, I., Silva-Espinoza, B.A., Ortega-Ramirez, L.A., Leyva, J.M., Siddiqui, M.W., Cruz-Valenzuela, M.R., Gonzalez-Aguilar, G.A., Ayala-Zavala, J.F., 2016. Oregano Essential Oil as an Antimicrobial and Antioxidant Additive in Food Products. Crit. Rev. Food Sci. Nutr. 56 (10), 1717–1727. https://doi.org/10.1080/10408398.2013.800832.
Roohinejad, S., Koubaa, M., Barba, F., Leong, S., A., K, Greiner, R., Chemat, F., 2017. Extraction Methods of Essential Oils from Herbs and Spices. Essential Oils in Food Processing: Chemistry, Safety and Applications. Wiley, pp. 21–55.
Ruan, E., Aalhus, J., Juárez, M., Sabik, H., 2015. Analysis of volatile and flavor compounds in grilled lean beef by stir bar sorptive extraction and thermal desorption—gas chromatography mass spectrometry. Food Anal. Methods 8. https://doi.org/10.1007/s12161-014-9881-z.

Sereshti, H., Samadi, S., 2014. A rapid and simple determination of caffeine in teas, coffees and eight beverages. Food Chem. 158, 8–13. https://doi.org/10.1016/j.foodchem.2014.02.095.

Shahina, Z., Al Homsi, R., Price, J.D.W., Whiteway, M., Sultana, T., Dahms, T.E.S., 2022. Rosemary essential oil and its components 1,8-cineole and α-pinene induce ROS-dependent lethality and ROS-independent virulence inhibition in *Candida albicans*. PloS One 17 (11), e0277097. https://doi.org/10.1371/journal.pone.0277097.

Silvosa, M., García-Moreno, M.D., Castro, R., 2025. Volatile sulphur compounds in wine distillates by stir bar sorptive extraction-gas chromatography-mass spectrometry. Appl. Sci. 15 (7). https://doi.org/10.3390/app15073680.

Singh, M., Singh, S., Mishra, S., Shankar, U., Maurya, A., Verma, R., 2024. Recent Advances in Extraction, Analysis, Value Addition, and Applications of Essential Oils. Medicinal and Aromatic Plants; Sustainable Landscape Planning and Natural Resources Management. Springer Nature, Cham, Switzerland, pp. 75–96. https://doi.org/10.1007/978-3-031-60117-0_7.

de Siqueira, A.C.P., Dutra Sandes, R.D., Nogueira, J.P., Araujo, H.C.S., de Jesus, M.S., Rajkumar, G., Leite Neta, M.T.S., Narain, N., 2024. Volatile profiles of Murcott and Ponkan mandarins obtained by stir bar sorptive extraction technique and their contributions to the fruit aroma. J. Food Sci. 89 (8), 4823–4838. https://doi.org/10.1111/1750-3841.17232.

Soleimani, M., Porgham Daryasari, A., Hejri, O., Mazaheri, R., 2014. Analysis of the volatile compounds in *Thymus vulgaris L.* using improved HS-SPME-GC-MS and comparison with conventional methods. J. Essent. Oil Bear. Plants 17. https://doi.org/10.1080/0972060X.2014.958548.

Song, G., Guo, X., Li, Q., Liao, J., Wang, D., Yuan, T., Li, L., Fang, R., Zhang, M., Shen, Q., Zheng, F., Gong, J., 2023. Simultaneous determination of various heavy metal and arsenic ions in seafood using functionalized fibrous silica (KCC-1) coated stir bar sorptive extraction prior to inductively coupled plasma mass spectrometry. Food Control 152, 109846. https://doi.org/10.1016/j.foodcont.2023.109846.

Tekin, K., Akalın, M.K., Şeker, M.G., 2015. Ultrasound bath-assisted extraction of essential oils from clove using central composite design. Ind. Crops Prod. 77, 954–960. https://doi.org/10.1016/j.indcrop.2015.09.071.

Tsakni, A., Chatzilazarou, A., Tsakali, E., Tsantes, A.G., Van Impe, J., Houhoula, D., 2023. Identification of bioactive compounds in plant extracts of greek flora and their antimicrobial and antioxidant activity. Separations 10 (7). https://doi.org/10.3390/separations10070373.

Vassiliou, E., Awoleye, O., Davis, A., Mishra, S., 2023. Anti-inflammatory and antimicrobial properties of thyme oil and its main constituents. Int. J. Mol. Sci. 24 (8). https://doi.org/10.3390/ijms24086936.

Vo, P., Phan, T., Luu, D., Tran, T., Pham, N., Ho, T., Ha, N., Nguyen, M., Nguyen, Q., 2024. Optimization of ultrasonic-assisted extraction of phenolics and terpenoids from sweet basil leaves using natural deep eutectic solvents. Int. J. Chem. Eng. 2024. https://doi.org/10.1155/2024/5199884.

Wejnerowska, G., Narloch, I., 2023. Comparison of the greenness assessment of chromatographic methods used for analysis of UV filters in cosmetic samples. Analytica 4 (4), 447–455. https://doi.org/10.3390/analytica4040032.

Wesołowska, A., Jadczak, D., 2019. Comparison of the chemical composition of essential oils isolated from two thyme (*Thymus vulgaris L.*) cultivars. Not. Bot. Horti Agrobot. Cluj. Napoca 47. https://doi.org/10.15835/nbha47311451.

Wojnowski, W., Tobiszewski, M., Pena-Pereira, F., Psillakis, E., 2022. AGREEprep – analytical greenness metric for sample preparation. TrAC Trends Anal. Chem. 149, 116553. https://doi.org/10.1016/j.trac.2022.116553.

Zhang, Q., Yao, X., He, M., Chen, B., Zhu, N., Hu, B., 2023. Porous aromatic framework/polydimethylsiloxane coated stir bar sorptive extraction coupled with high performance liquid chromatography-diode array detection of trace polychlorinated biphenyls analysis in environmental waters. J. Chromatogr. A 1688, 463709. https://doi.org/10.1016/j.chroma.2022.463709.

Zhao, J., Quinto, M., Zakia, F., Li, D., 2023. Microextraction of essential oils: a review. J. Chromatogr. A 1708, 464357. https://doi.org/10.1016/j.chroma.2023.464357.

Zhong, C., He, M., Liao, H., Chen, B., Wang, C., Hu, B., 2016. Polydimethylsiloxane/covalent triazine frameworks coated stir bar sorptive extraction coupled with high performance liquid chromatography-ultraviolet detection for the determination of phenols in environmental water samples. J. Chromatogr. A 1441, 8–15. https://doi.org/10.1016/j.chroma.2016.02.073.