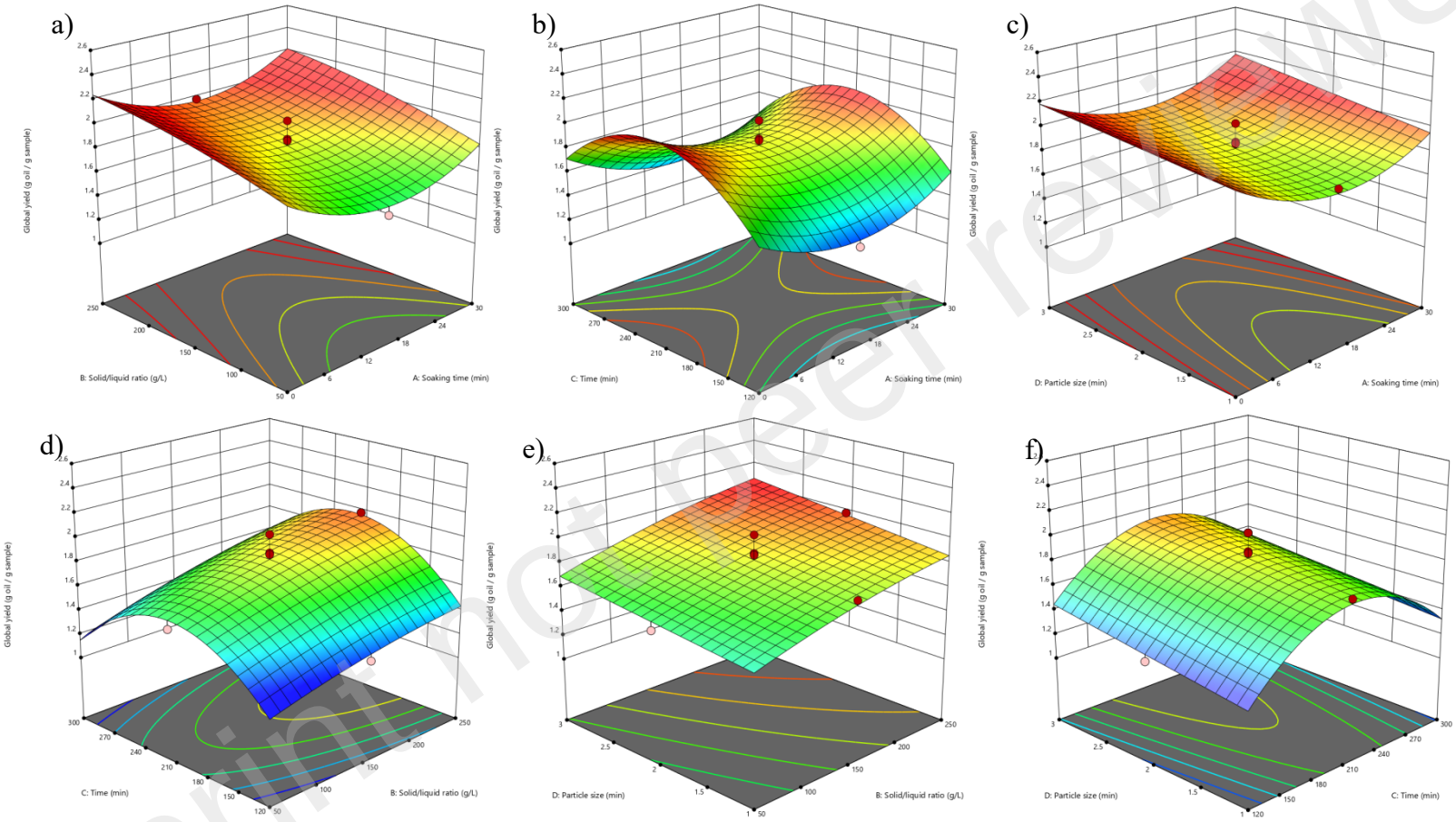

Figure 1 - Response surface plots obtained for EO yield ($Y1_{HD}$) of RO: a) solid/liquid ratio *vs.* soaking time; b) extraction time *vs.* soaking time; c) particle size *vs.* soaking time; d) extraction time *vs.* solid/liquid ratio; e) particle size *vs.* solid/liquid ratio; f) particle size *vs.* extraction time.

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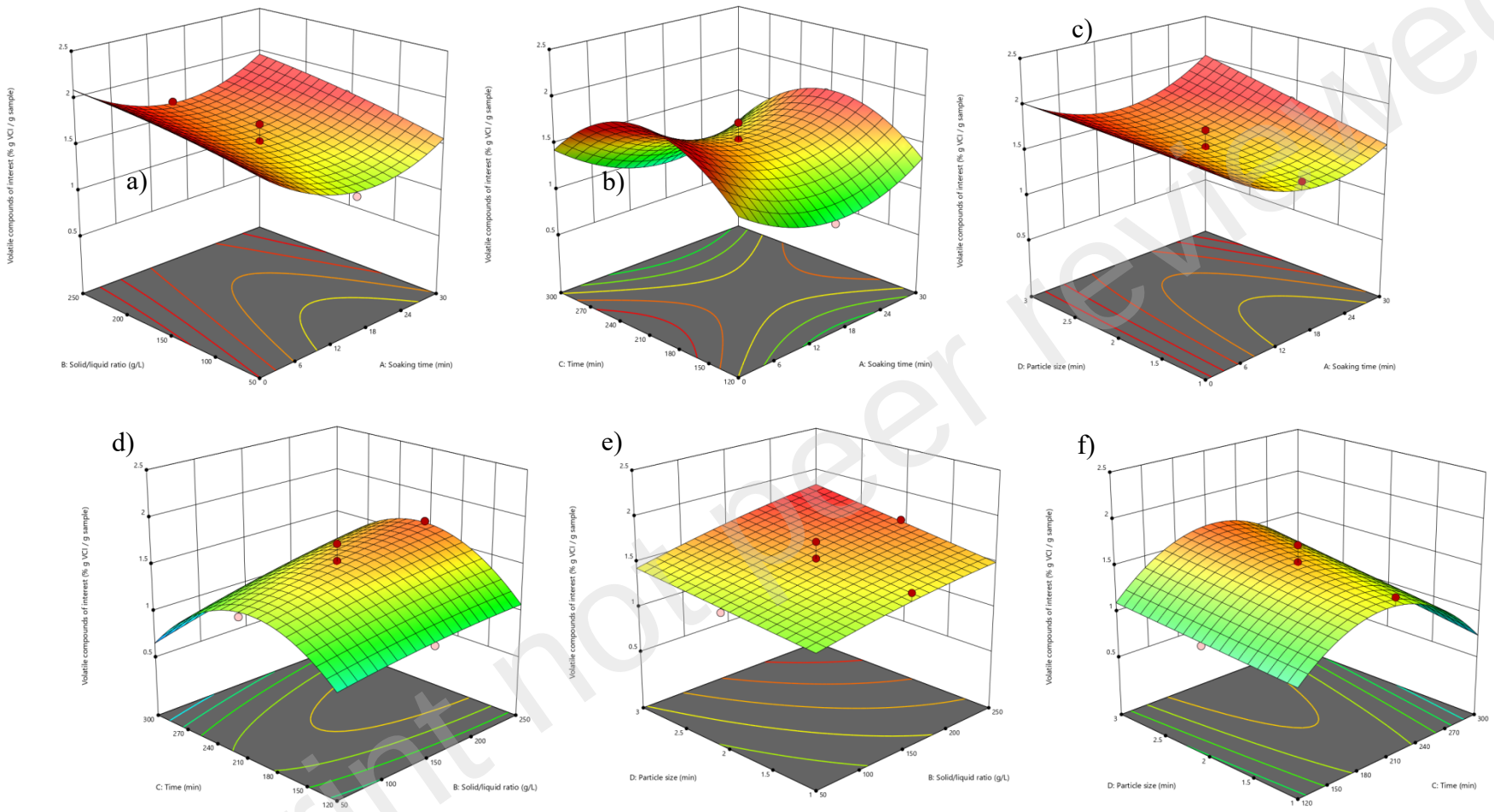

Figure 2 - Response surface plots obtained for VCI ($Y2_{HD}$) of RO: a) solid/liquid ratio *vs.* soaking time; b) extraction time *vs.* soaking time; c) particle size *vs.* soaking time; d) extraction time *vs.* solid/liquid ratio; e) particle size *vs.* solid/liquid ratio; f) particle size *vs.* extraction time.

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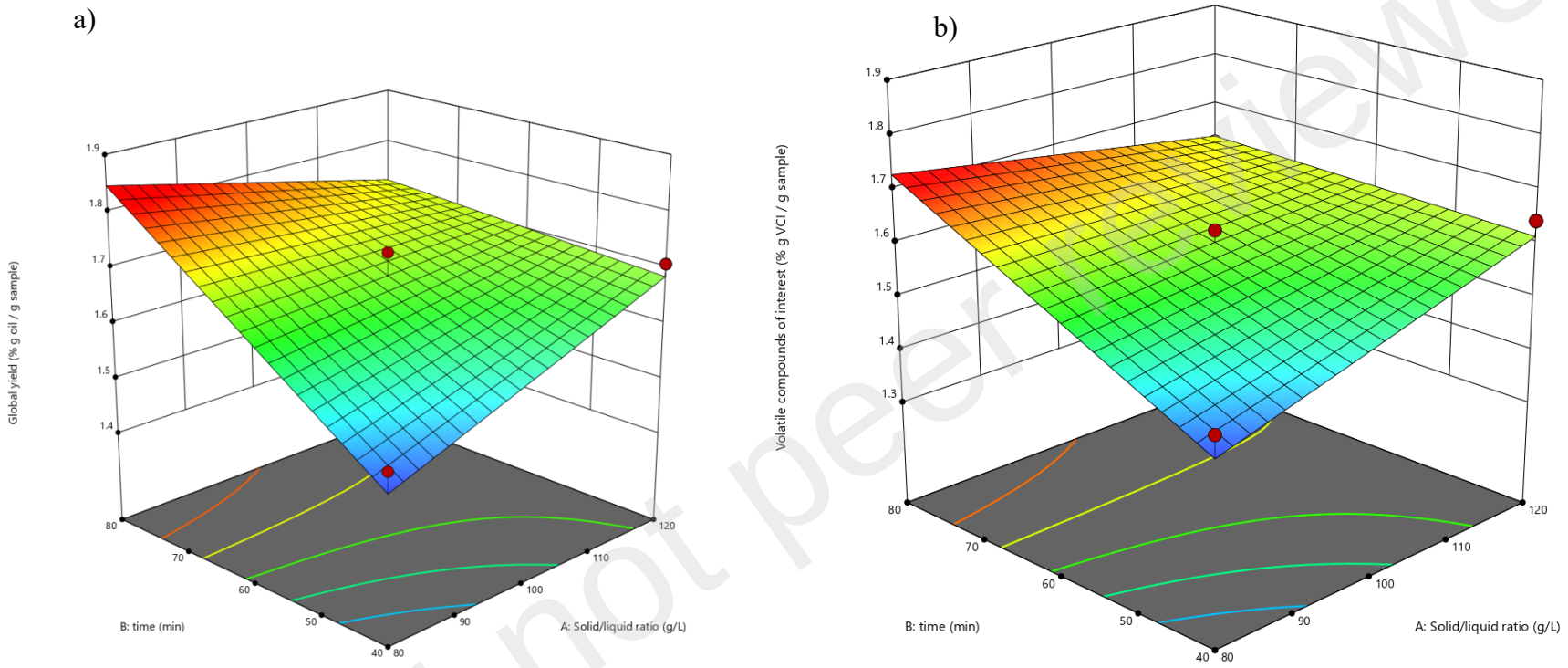

Figure 3 - Response surface plots of time *vs.* solid/liquid ratio obtained for a) EO yield, and b) VCI of RO through MAHD.

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Traditional *vs* microwave-assisted hydrodistillation of *Rosmarinus officinalis* L. essential oil: a response surface optimization approach

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Abstract

Essential oils (EOs) from rosemary (*Rosmarinus officinalis* L.) were optimized as natural preservatives using hydrodistillation (HD) and microwave-assisted hydrodistillation (MAHD) with response surface methodology and multi-response desirability analysis.

For HD, a Box–Behnken design assessed soaking time, particle size, solid/liquid ratio, and extraction time. MAHD optimization used a two-stage approach: fractional factorial screening followed by a central composite design focusing on solid/liquid ratio and extraction time. Responses included EO yield and volatile compound content (VCI).

Both quadratic (HD) and two-factor interaction (MAHD) models showed strong fit (R^2 up to 0.95) with no significant lack of fit. HD produced higher yields and VCI at longer extraction times, while MAHD achieved faster extraction with slightly lower yields but improved consistency in oxygenated monoterpenes. GC–MS confirmed a chemotype dominated by 1,8-cineole, camphor, borneol, α-terpineol, and α-pinene.

Keywords: aromatic plants; essential oil; volatile compounds; gas chromatography.

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1. INTRODUCTION

Essential oils (EO) are complex mixtures of volatile secondary metabolites, mainly produced by aromatic plants. They contribute to plant defence, pollinator attraction, and abiotic stress tolerance, and have become important as natural antimicrobials, flavorings, fragrances, antioxidants, and functional ingredients for food, cosmetic, and pharmaceutical industries (Rathore et al., 2022). Rosemary (*Rosmarinus officinalis* L.) is one of the primary commercial sources of EO, which is typically rich in monoterpenes and oxygen-containing monoterpenes, such as 1,8-cineole, camphor, α-pinene, camphene, and borneol, and, together with non-volatile phenolics like carnosic acid and rosmarinic acid, has a range of bioactive activities, including antioxidant, antimicrobial, and anti-inflammatory (Guelifet et al., 2025; Teshale et al., 2022).

Traditionally, EOs are obtained by conventional hydrodistillation (HD) or steam distillation, processes that are simple, robust, and easily scalable, that remain the standard methods. HD uses boiling water to rupture cell structures and drag volatiles with the vapor phase but typically requires long extraction times (often 2–4 h), substantial energy input, and large water volumes, which can promote hydrolysis and thermal degradation (Moradi et al., 2018; Taheri et al., 2023). In contrast, non-conventional techniques, such as microcrowave-assisted hydrodistillation (MAHD), have been proposed as greener alternatives, aiming to shorten extraction time, reduce energy consumption, and improve the preservation of bioactive volatiles (Bousbia et al., 2009; Guelifet et al., 2025). Microwave-based methods exploit the heating of the water or solvent in the sample, resulting in a rapid increase in temperature and pressure, which can rupture the cell and accelerate the mass transfer of the essential oil to the vapour phase. However, they may require careful control of power and process conditions to avoid overheating, structural collapse, or over-decomposition of thermolabile constituents (Moradi et al., 2018).

Given the influence of processing conditions on both yield and composition, optimization is essential for designing rosemary EO extraction processes that balance productivity and quality. Response surface methodology (RSM) allows simultaneous evaluation of multiple factors such as temperature, extraction time, sample, solid–liquid ratio, and power, and their interactions, using a limited number of experiments. Beyond individual responses, the desirability function provides a powerful tool for combining multiple responses into a single global objective. In this context, individual desirability functions can be defined for yield, targeted high-quality volatiles, and functional outputs

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(e.g., antioxidant or antimicrobial activities), along with the global desirability. By maximizing global desirability, it becomes possible to identify operating conditions that balance high oil recovery with enrichment in bioactive compounds and acceptable process efficiency, rather than optimizing yield at the expense of quality or vice versa (Elyemni et al., 2021; Teshale et al., 2022).

In the food sector, rosemary extracts are already authorized as natural antioxidants in food and beverages in European Union, where a high content of oxygenated monoterpenes and phenolic diterpenes is linked to effective delay of lipid oxidation in food systems, and edible coatings, frequently allowing the partial replacement of synthetic antioxidants (Nieto et al., 2018; Vital et al., 2016). Optimized extraction processes that improve the reproducibility of volatile profiles also facilitate their use as natural antimicrobial or antioxidant additives in active packaging films and as bioactive payloads in green nanotechnology platforms, where consistent composition and scalable, energy-efficient extraction are prerequisites for industrial translation (Shahrampour & Razavi, 2023). This study brings a distinct contribution by applying a set of design of experiments to compare HD and MAE, integrating both overall yield and targeted fraction of bioactive volatile compounds into a multi-response desirability function. In addition, a strategy combining fractional factorial screening and RSM is implemented for MAHD, enabling a focused exploration of the most influential variables.

Therefore, the present study addresses the optimization and evaluation of conventional HD and MAHD extraction, applying response surface methodology with a unified response and desirability function to obtain high-quality rosemary EO.

2. MATERIAL AND METHODS

2.1. Plant material and reagents

Rosemary (*Rosmarinus officinalis* L.) leaves were obtained from the commercial supplier “Rota das Índias” (Lisbon, Portugal). The plant material was ground in a Thermomix (Vorwerk Bimby TM6, Carnaxide, Portugal) and sieved into three distinct particle sizes (<1 mm, 2 mm, and 3.5 mm). The fractions were stored under dry, cool, and light-protected conditions until further use. All chemicals and reagents used in this study

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were of analytical grade and, whenever necessary, of high-performance liquid chromatography (HPLC) grade.

2.2. Hydrodistillation (HD)

Conventional extraction of EOs was performed by HD in a Clevenger-type apparatus. Rosemary samples were weighed and extracted with distilled water. The four variables defined were: sample solid/liquid ratio (g/L), soaking time (min), particle size (mm), and extraction time (min), which were set according to the designed runs. All experiments were carried out in a 1 L round-bottom flask containing 400 mL of distilled water. The extraction period was recorded from the moment of the first oil droplet in the Clevenger apparatus. At the end of the distillation, the hydrolate was discarded, and the EO was transferred to a pre-weighed vial for yield determination based on the mass of recovered EO. The vials were stored at −20 °C until further analysis.

2.2.1. Experimental design for Response Surface Methodology (RSM)

The optimization of EO extraction from rosemary using HD was performed using RSM. Two response variables were considered: the extraction yield ($Y1_{HD}$: % $_{oil}$/g $_{sample}$) and the quantity of volatile compounds of interest (VCI) ($Y2_{HD}$: % $_{vci}$/g $_{sample}$). A Box-Behnken design (BBD) comprising four independent variables: soaking time ($X1_{HD}$: 0-30 min), particle size ($X2_{HD}$: 1–3 mm), solid/liquid ratio ($X3_{HD}$: 50–250 g/L), and extraction time ($X4_{HD}$: 120–300 min), each evaluated at three coded levels, was implemented. The experimental conditions were selected based on previous studies (Conde-Hernández et al., 2017; Gonzalez-Rivera et al., 2023; Teshale et al., 2022; Walid et al., 2019) and subsequently refined through preliminary trials.

Among the identified compounds, the VCIs (1,8-cineol, camphor, borneol, bornyl acetate, linalool, *α*-terpineol, *α*- and *β*-pinene, camphene, and *p*-cymene) were chosen based on their reported bioactive activities, particularly antioxidant and/or antimicrobial potential (Aragão et al., 2026; Conde-Hernández et al., 2017; Taheri et al., 2023; Teshale et al., 2022). Therefore, VCI values represent the sum of these volatiles. Individual models were developed for each response, and global desirability functions were established to achieve the optimization objectives: maximizing both extraction yield and

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the abundance of the VCIs. The final optimization step was to maximize the overall desirability index, integrating both responses to determine the optimal HD parameters. Data analysis and model development were performed using Design Expert software version 13 (Stat-Ease Inc., Minneapolis, MN, USA).

2.3. Microwave-assisted hydrodistillation (MAHD)

For the non-conventional extraction of EOs, a microwave synthesis/extraction system (NuWay-uno, Nutech Analytical Technologies Pvt. Ltd., Kolkata, India) coupled to a Clevenger-type apparatus (MAHD) was used. The overall procedure followed a similar setup to conventional HD, differing mainly in the heat source, which in this case was microwave irradiation, as well as in the specific operating conditions used. The tested variables were solid/liquid ratio (g/L), extraction time (min), particle size (mm), and microwave power (W). All experiments were carried out in a 1 L microwave flask, containing 400 mL of distilled water. After extraction, the EO was collected in pre-weighed vials, and the extraction yield was calculated. The vials were stored at −20 °C until further analysis.

2.3.1. Screening and central composite design for MAHD

A distinct strategy was adopted for the MAHD process to minimize the number of experimental runs and enhance the efficiency of the RSM. First, a screening stage was performed using a two-level fractional factorial design with resolution IV (2^{4-1}) implemented in Design Expert to identify variables with significant influence on the extraction. The four factors were solid/liquid ratio ($X1_{MAHD}$: 50-100 g/L), time ($X2_{MAHD}$: 10-60 min), microwave power ($X3_{MAHD}$: 200-500 W), and soaking time (X4: 0-30 min), considering only the extraction yield per unit mass ($Y1_{MAHD}$: g_{oil}) as the response. The parameter ranges were also selected based on previous literature (Bousbia et al., 2009; Conde-Hernández et al., 2017; Elyemni et al., 2021; Gonzalez-Rivera et al., 2023). The screening results showed that two variables, solid/liquid ratio and extraction time, had a significant effect on the process. Consequently, a response surface approach was applied, using a Central Composite Circumscribed Design (CCC), with two significant factors, solid-to-liquid ratio ($X1_{MAHD}$: 80-120 g/L) and extraction time ($X2_{MAHD}$: 40-80 min),

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each at three levels (-1, 0, +1). The factorial portion was expanded with axial points (±α, α ≈ 1.414), with the corresponding values for the axial points being 71.72 and 128.28 g/L for solid/liquid ratio, and 31.72 and 88.28 min for extraction time. The final design consisted of 4 factorial points, 4 axial points, and 5 replicated center points, giving a total of 13 experimental runs.

The response variables were extraction yield ($Y1_{MAHD}$: % $_{oil}$/g $_{sample}$) and VCI content ($Y2_{MAHD}$: % $_{vci}$/g $_{sample}$). As in the HD experiments, RSM was used to achieve the global desirability function and to maximize both responses, thereby identifying the optimal MAHD extraction conditions.

2.4. Gas-chromatography

The EO volatiles were identified through gas chromatography according to Sprea et al. (2023). A GC-2010 Plus gas chromatography system equipped with an AOC-20i Plus autosampler (Shimadzu, Kyoto, Japan), a quadrupole mass spectrometer detector, and an SH-RXi-5ms capillary column (30 m × 0.25 mm × 0.25 µm; Shimadzu, Columbia, SC, USA) was used. The temperature program was set to 45–175 °C at 3 °C min^{-1}, followed by a ramp of 15 °C min^{-1} to 300 °C, then held isothermal for 10 min. The injector temperature was maintained at 280 °C, and 1 µL of sample was injected. The transfer line and ion source temperatures were 280 °C and 220 °C, respectively. Helium was used as the carrier gas, adjusted to a linear velocity of 30 cm·s^{-1}, with a split ratio of 1:20, an ionization energy of 70 eV, a mass scan range of 40–300 u, and a scan time of 1 s. Compound identification was based on comparison of mass spectra with the NIST17 library (similarity > 90%), complemented by calculation of linear retention indices (LRI) relative to an *n*-alkane series (C8–C40, ref. 40147-U, Supelco) and comparison with published reference data (Adams, 2017), and, whenever available, with commercial standard compounds. Quantification was performed as relative percentage of total volatiles, using the peak areas derived from the total ion current (TIC). All analyses were run in duplicate.

2.5. Statistical analysis

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All results were reported as mean ± standard deviation (SD). The models generated from the experimental designs were evaluated by analysis of variance (ANOVA). Data processing and statistical analyses were carried out using Design-Expert 13, with a significance level of 0.05 (p-value = 0.05).

3. RESULTS AND DISCUSSION

3.1. Comparison of HD and MAHD volatile profiles

Following the extraction of all samples from the design, the EOs were injected into the GC-MS for the identification and quantification of volatile compounds. **Table 1** exhibits the range of values for the volatile profile of the rosemary EO obtained by HD and MAHD while **Table 2** shows the range of values for the major classes of volatile compounds.

Table 1 – Volatile profile of rosemary EO obtained by HD and MAHD. Results expressed in relative area percentages (%).

Peak	LRI[a]	LRI[b]	Volatile compound	Area range (%)	
				HD	MAE
1	921	919	Tricyclene	0.03 - 0.17	0.11 - 0.16
2	924	925	*α*-Thujene	0.01 - 0.05	-
3	932	931	*α*-Pinene	2.88 - 11.81	8.24 - 10.32
4	946	945	Camphene	1.18 - 4.27	3.47 - 4.35
5	953	951	Thuja-2,4(10)-diene	0.02 - 0.11	0.02 - 0.02
6	974	973	*β*-Pinene	0.82 - 2.42	1.43 - 2.06
7	983	980	1-Octen-3-ol	0.02 - 0.26	0.2 - 0.31
8	979	986	Octanone	ni - 0.09	0.04 - 0.09
9	988	990	*β*-Myrcene	0.96 - 1.51	1.06 - 1.66
10	1005	1002	*α*-Phellandrene	0.13 - 0.26	0.13 - 0.21
11	1008	1008	*δ-3*-Carene	0.01 - 0.07	-
12	1014	1015	*α*-Terpinene	0.36 - 0.72	0.36 - 0.51
13	1022	1024	*p*-Cymene	2.76 - 3.97	3.03 - 3.72
14	1026	1032	1,8-Cineole	34.48 - 46.55	34.53 - 38.66
15	1044	1037	(*E*)-*β*-Ocimene	0.01 - 0.09	-
16	1054	1057	*γ*-Terpinene	0.12 - 0.49	0.13 - 0.22
17	1065	1065	*cis*-Sabinene hydrate	0.01 - 0.09	0.02 - 0.03
18	1085	1087	Mentha-2,4(8)-diene	0.14 - 0.46	0.14 - 0.19
19	1095	1099	Linalool	1.26 - 2.4	1.89 - 2.21

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20	1114	1112	Fenchol	0.14 - 0.25	0.21 - 0.25
21	1118	1120	*cis*-2-*p*-Menthen-1-ol	0.05 - 0.11	0.05 - 0.08
22	1122	1124	Campholenal	0.08 - 0.19	0.11 - 0.13
23	1141	1144	Camphor	12.4 - 18.53	17.9 - 19.55
24	1148	1154	Menthone	ni - 0.19	-
25	1144	1158	*iso*-Isopulegol	0.05 - 0.17	0.16 - 0.2
26	1158	1162	*trans*-Pinocamphone	0.02 - 0.44	-
27	1160	1169	Pinocarvone	0.02 - 0.41	0.02 - 0.04
28	1165	1175	Borneol	5.11 - 8.7	7.81 - 8.75
29	1174	1184	Terpinen-4-ol	1.27 - 2.81	1.77 - 2.05
30	1179	1190	*ρ*-Cymen-8-ol	0.07 - 0.32	0.14 - 0.2
31	1186	1199	*α*-Terpineol	5.3 - 10.43	6.59 - 7.72
32	1204	1251	Verbenone	0.02 - 1.68	-
33	1284	1293	Bornyl acetate	0.3 - 1.01	0.67 - 0.93
34	1417	1437	*β*-Caryophyllene	ni - 1.87	0.68 - 1.26
35	1452	1506	*α*-Humulene	ni - 0.22	0.08 - 0.19
36	1582	1601	Caryophyllene oxide	0.02 - 0.55	0.22 - 0.44
37	1608	1629	Humulene epoxide II	0.22 - 1.01	0.04 - 0.1
38	1632	1638	Humulenol-II	0.06 - 0.37	0.09 - 0.23
39	1643	1644	Caryophylla-4(12),8(13)-dien-5-*β*-ol	-	0.17 - 0.36
			Total identified	**92.59 - 98.42**	**98.15 - 99.05**
			Not identified	**1.58 - 7.41**	**0.95 - 1.85**

[a]: calculated LRI, determined against a series of n-alkanes(C7-C40);

[b]: theoretical LRI, according to the literature (Adams, 2017).

Table 2 – Major classes of identified volatile compounds in rosemary EO obtained by HD and MAHD.

Major classes	HD (%)	MAHD (%)
Monoterpene hydrocarbons	7.0 - 21.4	18.39 - 23.27
Oxygen-containing monoterpenes	70.56 - 81.86	72.48 - 79.09
Sesquiterpene hydrocarbons	0.01 - 1.96	0.76 - 1.45
Oxygen-containing sesquiterpenes	0.32 - 1.59	0.53 - 1.11
Others	0.1 - 0.38	0.21 - 0.34

A total of 38 and 33 compounds were identified and quantified in the EO obtained by HD and MAHD, respectively, with *α*-thujene, *δ-3*-carene, (*E*)-*β*-ocimene, menthone, *trans*-pinocamphone, and verbenone being detected only in HD EO, while caryophylla-4(12),8(13)-dien-5-*β*-ol was only detected in MAHD. Overall, HD samples had a higher content of unidentified constituents (1.58-7.41% HD *vs*. 0.95-1.85% MAHD). In

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literature, across many methods, GC–MS analyses consistently showed typical rosemary chemotypes, dominated by monoterpene hydrocarbons and oxygenated monoterpenes such as *α*-pinene, camphene, 1,8-cineole (eucalyptol), camphor, borneol, *α*-terpineol and verbenone (most bioactive fraction), with smaller fractions of sesquiterpenes (e.g., *β*-caryophyllene, *α*-humulene) and their oxides, which contribute to aroma and bioactivity. However, microwave-based methods systematically enriched the fraction of oxygenated monoterpenes compared with HD (Bousbia et al., 2009; Halimi et al., 2025; Taheri et al., 2023).

HD and MAHD produced oils with very similar qualitative profiles, but with apparent quantitative differences in key constituents and chemical groups. Oxygenated monoterpenes dominated in both extracts, but HD showed a slightly broader range (70.56-81.86%) compared with MAHD (72.48-79.09%), whereas monoterpene hydrocarbons were consistently higher using MAHD (18.39-23.27%) as compared with HD (0.59-21.40%). For major individual compounds, *α*-pinene, 1,8-cineole, camphor, borneol and *α*-terpineol were abundant in both methods, with somewhat narrower, more stable ranges in MAHD (e.g., 1,8-cineole 34.53–38.66% vs. 34.48–46.55% in HD; camphor 17.90–19.55% vs. 12.40–26.98% in HD). Sesquiterpene hydrocarbons and oxygenated sesquiterpenes appeared at low levels in both cases, with slightly higher minimum values in MAHD, but these differences are minor compared with the monoterpene fraction. Overall, MAHD produces an EO that is more consistent in key oxygenated monoterpenes such as 1,8-cineole, camphor, borneol, and *α*-terpineol. In contrast, HD shows greater variability in the number of compounds, which could be of interest for aromatic applications, where subtle flavour nuances are valued(Chen et al., 2024). Reported literature ranges for rosemary EO are often wide, with 1,8-cineole varying between 10.2% to 51.8%, *α*-pinene 14.1% to 51.5%, camphor 5.1% to 36.5% and borneol 0.5% to 15.4% (Guelifet et al., 2025; Kamli et al., 2017; Mollova et al., 2025; Moradi et al., 2018; Sakar et al., 2023). Variations in the volatile composition of rosemary EO may be the result of a combination of biological, environmental, and technological factors (Rathore et al., 2022; Sadeh et al., 2019). A hierarchical strategy involving a screening step offers a valuable alternative, as it enables a focused exploration of the most influential variables and yields more consistent profiles, as revealed in the MAHD design and further discussed. This consistency is advantageous for applications that require tight control over EO composition such as medicine fields (Xia et al., 2025).

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3.2. Model fitting and response surface analysis for HD

The optimization was based on two response variables: EO yield ($Y1_{HD}$), calculated from the mass of EO obtained, and VCI content ($Y2_{HD}$), calculated after identifying the sample's volatile profile. The responses value obtained in the RSM are shown in **Table 3**. **Error! Reference source not found.** and **Error! Reference source not found.** illustrate the response surfaces for EO extraction from rosemary by HD for $Y1_{HD}$ and $Y2_{HD}$, respectively.

Table 3 – RSM design and the respective response values for EO yield and VCI by HD.

Run	Soaking time (min)	Particle Size (mm)	Liquid/Solid ratio (g/L)	Extraction time (min)	Yield (% oil/ g sample)	VCI (% VCI/ g sample)
1	0	1	50	120	1.2876	0.9860
2	0	1	50	300	1.3824	1.1349
3	0	1	250	120	1.6324	1.4036
4	0	1	250	300	1.7864	1.5016
5	0	3	50	120	1.5412	1.2993
6	0	3	50	300	1.5840	1.2736
7	0	3	250	120	1.7750	1.5317
8	0	3	250	300	1.9302	1.6892
9	30	1	50	120	1.3416	1.1089
10	30	1	50	300	0.6428	0.5016
11	30	1	250	120	1.4999	1.1801
12	30	1	250	300	1.6951	1.0734
13	30	3	50	120	1.5520	1.3333
14	30	3	50	300	1.4340	1.1566
15	30	3	250	120	1.9014	1.6466
16	30	3	250	300	1.9557	1.7302
17	0	2	150	210	1.8725	1.6146
18	30	2	150	210	2.0145	1.7702
19	15	1	150	210	1.7713	1.5076
20	15	3	150	210	1.7008	1.4904
21	15	2	50	210	1.5352	1.3003
22	15	2	250	210	1.9814	1.6823
23	15	2	150	120	1.2829	1.0035
24	15	2	150	300	1.7755	1.4289
25	15	2	150	210	1.8817	1.5145
26	15	2	150	210	1.8683	1.5478
27	15	2	150	210	2.0327	1.7313

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FIGURE1

FIGURE2

The reduced quadratic models for EO yield and VCI showed reasonable statistical quality and were suitable for optimization. For $Y1_{HD}$, the model was significant (F-value = 6.66, $p = 0.0025$), with a non-significant lack-of-fit (p-value = 0.4117), and $R^2 = 0.8965$ and adjusted $R^2 = 0.7619$, indicating considerable explanatory power for the predictive model. Moreover, an Adeq. Precision of 8.7929 was obtained, which is above the recommended minimum of 4. Solid/liquid ratio ($X2_{HD}$: p-value < 0.0001) and particle size ($X4_{HD}$: p-value = 0.0092) were the significant linear factors, and soaking time ($X1_{HD}^2$: p-value < 0.0186) and time ($X3_{HD}^2$: p-value = 0.0007) were the significant quadratic terms in the model.

In contrast, the model for $Y2_{HD}$ showed stronger performance, with $R^2 = 0.9469$, adjusted $R^2 = 0.8779$, and an adequate precision of 15.5521, indicating superior predictive ability for this response. The model was significant (F-value = 13.72, $p < 0.0001$), with a non-significant lack-of-fit (p-value = 0.6894). Soaking time ($X1_{HD}$: p-value = 0.0052), solid/liquid ratio ($X2_{HD}$: p-value < 0.0001) and particle size ($X4_{HD}$: p-value = 0.0007) were the significant linear factors, while the two-factor interaction terms soaking time*particle size ($X1_{HD}$*$X4_{HD}$: p-value = 0.0036) and solid/liquid ratio*extraction time ($X2_{HD}$*$X3_{HD}$: p-value = 0.0128) significant, along with the quadratic terms $X1_{HD}^2$ (p-value = 0.0017) and $X3_{HD}^2$ (p-value < 0.0001), which shows a good ability to explain the variation in response. Comparing the models for both responses, the VCI model suggests superior predictive performance for this response.

For both responses, the diagnostic plots (normal probability of residuals, residuals *vs.* predicted values, and Cook's distance) showed that residuals were approximately normal, variance was constant with no clear trend or funnel pattern, and there were no influential outliers. Thus, supporting the validity of the predictive models and the use of the desirability function for the simultaneous optimization of $Y1_{HD}$ and $Y2_{HD}$.

3.3. Model fitting and response surface analysis for MAHD

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The optimization of the MAHD process was performed in two sequential stages. First, a two-level factorial screening design with four factors was performed to identify variables that were significant to the process, followed by an RSM CCC using only the two significant factors from the screening step. In the RSM, the responses considered were the EO yield ($Y1_{MAHD}$) and VCI ($Y2_{MAHD}$), consistent with the variables evaluated for the HD process. The experimental matrix and the yields obtained in the screening phase are presented in **Table 4**.

Table 4 – Two-level factorial experimental design and corresponding EO weight responses for MAHD.

Run	Solid/liquid ratio (g/L)	Extraction time (min)	Microwave power (W)	Soaking time (min)	EO weight (mg)
1	50	10	200	0	110.0
2	50	60	500	0	240.8
3	50	60	200	30	191.6
4	50	60	200	30	165.4
5	100	10	200	30	312.3
6	100	10	500	0	300.8
7	50	10	500	30	53.9
8	100	10	200	30	312.0
9	50	10	500	30	56.4
10	50	10	200	0	63.8
11	100	60	500	30	419.7
12	100	10	500	0	333.8
13	100	60	200	0	608.4
14	100	60	200	0	664.4
15	100	60	500	30	596.6
16	50	60	500	0	208.5

The selected factorial model was highly significant ($F = 124.35$, p-value < 0.0001), with linear effects of solid/liquid ratio ($F = 262.92$, p-value < 0.0001), extraction time ($F = 102.87$, p-value < 0.0001), and soaking time ($F = 7.27$, p-value $= 0.0195$) contributing significantly to the transformed yield (Box–Cox square-root transformation). The lack of fit was not significant (p-value $= 0.7018$). The quality of fit was excellent, as indicated by high coefficients of determination ($R^2 = 0.9688$, adjusted $R^2 = 0.9610$, predicted $R^2 = 0.9446$) and an Adeq. Precision of 29.0530, far above the recommended minimum. Residual diagnostics (normal probability plot, residuals *vs.* predicted values, Box–Cox

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curve, and Cook's distance) confirmed that the model assumptions were satisfied, supporting the use of this factorial model to identify the most influential variables and guide subsequent response surface optimization. Therefore, two significant variables were chosen for the RSM, with soaking time excluded because it had the least influence on variability. **Table 5** summarises the response values obtained from the CCC runs, and **Error! Reference source not found.** shows the response surface obtained for EO yield and VCI from rosemary.

FIGURE3

Table 5 – RSM design and the corresponding response values obtained using MAHD.

Run	Solid/liquid ratio (g/L)	Extraction time (min)	Yield (% oil/g sample)	VCI (% VCI/g sample)
1	100	60	1.6828	1.5984
2	100	60	1.6645	1.5858
3	100	60	1.5640	1.4867
4	128	60	1.7305	1.6475
5	100	60	1.2138	1.1438
6	80	80	1.6900	1.5913
7	100	31	1.4883	1.4321
8	120	80	1.7123	1.6239
9	100	60	1.7293	1.6267
10	80	40	1.5309	1.4779
11	71	60	1.6600	1.5714
12	120	40	1.7088	1.6439
13	100	88	1.8170	1.7100

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The CCC generated statistically significant 2FI models for both $Y1_{MAHD}$ and $Y2_{MAHD}$. For $Y1_{MAHD}$, the model was significant (F-value = 17.97, *p*-value = 0.0021), with a non-significant lack-of-fit (*p*-value = 0.4566), a R^2 = 0.8998, adjusted R^2 = 0.8498 and predicted R^2 = 0.6543 and predictive power (Adeq. Precision = 13.9777), with significant effects from extraction time ($X2_{MAHD}$: F-value = 41.60, *p*-value = 0.0007) and solid/liquid ratio*time interaction ($X1_{MAHD}$*$X2_{MAHD}$: F-value = 10.66, *p*-value = 0.0171).

The model for $Y2_{MAHD}$ also was significant (F-value = 14.21, *p*-value = 0.0039), with a non-significant lack-of-fit (*p*-value = 0.2196), R^2 = 0.8766, adjusted R^2 = 0.8149 and predicted R^2 = 0.4877, and Adeq. Precision of 12.3708, driven by extraction time ($X2_{MAHD}$: F-value = 29.71, *p*-value = 0.0016) and solid/liquid ratio*time interaction ($X1_{MAHD}$*$X2_{MAHD}$: F-value = 9.08, *p*-value = 0.0236). Although its predictive capability was more moderate (difference between adjusted and predicted R^2 > 0.2), residual diagnostics (normal probability plots, residuals *vs*. predicted values, Box–Cox analyses without recommended transformations, and Cook's distance plots) confirmed that regression assumptions were reasonably met for both responses, validating their use with the multi-response desirability function for process optimization, with maximum yield and VCI, within the studied experimental domain.

3.4. Multi-response optimization through desirability functions

Because both the overall EO yield and the amounts of specific volatile constituents were considered critical responses, a desirability function approach was applied to identify conditions that simultaneously maximize both variables. A second-order polynomial regression was applied to the HD experimental results, expressed in coded units for soaking time (A), solid/liquid ratio (B), extraction time (C), and particle size (D). The individual model equations for yield ($Y1_{HD}$) and VCI ($Y2_{HD}$) were:

$$Y1_{HD} = +1.83 - 0.0206 * A + 0.1796 * B + 0.0349 * C + 0.0951 * D + 0.0081 * AB - 0.0244 * AC + 0.0187 * AD + 0.0384 * BC + 0.0072 * BD - 0.0146 * CD + 0.2626 * A^2 - 0.0422 * B^2 - 0.4546 * C^2$$

$$Y2_{HD} = +1.54 - 0.1001 * A - 0.1593 * B - 0.0543 * C + 0.1265 * D + 0.0359 * AB - 0.0443 * AC + 0.1070 * AD + 0.0856 * BC + 0.0365 * BD + 0.0614 * CD + 0.3620 * A^2 - 0.0349 * B^2 - 0.5592 * C^2$$

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The optimal HD setting predicted by the model corresponded to 200 min of extraction, a solid/liquid ratio of 144 g/L, particle size of 2 mm, and soaking time of 0 min, yielding 2.278% and 1.988% of VCI (22.78 and 19.88 mg/g, respectively), with an overall desirability value of 1.00.

The experimental data for MAHD were fitted to a linear model with two-factor interaction terms (2FI) for EO yield ($Y1_{MAHD}$) and VCI ($Y2_{MAHD}$) using coded variables E (solid/liquid ratio) and F (extraction time):

$$Y1_{\mathrm{MAHD}} = +1.69 + 0.0180 * \mathrm{E} + 0.0980 * \mathrm{F} - 0.0779 * \mathrm{EF}$$

$$Y2_{\mathrm{MAHD}} = +1.60 + 0.0211 * \mathrm{E} + 0.0780 * \mathrm{F} - 0.0677 * \mathrm{EF}$$

The optimal MAHD condition identified in this study corresponded to a solid/liquid ratio of 80 g/L, 80 min, and a power of 350W. Under these settings, the process afforded a global yield of 1.846%, with 1.729% of VCI, which is equivalent to 18.46 mg/g and 17.29 mg/g, respectively, and an overall desirability index of 1.00.

3.5. Comparison of HD and MAHD extraction methods for essential oil extraction

In terms of model fitting, the HD process provided an excellent fit for both yield and VCI, mainly for VCI ($R^2 = 0.95$, non-significant lack-of-fit, strong linear, interaction and quadratic terms). In contrast, MAHD provided simpler 2FI models with moderate descriptive power for both responses. The corresponding desirability analyses reflect these differences. For HD, the global desirability function identified an optimum at longer extraction times and higher solid loading, giving the highest absolute yield and VCI content. For MAHD, the optimum was reached at shorter extraction times and lower solid/liquid ratios, with only slightly lower yields.

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Comparing the two optimization strategies, the screening step before the MAHD RSM proved advantageous, as it allowed the response ranges for the volatile compounds to be more clustered, because of the narrower space factor This approach contributed to the shorter and more homogeneous ranges observed for volatiles in MAHD EO, providing a more precise definition of the optimal extraction window. In addition, the analysis of HD and MAHD for rosemary EO demonstrates that process intensification by microwave energy is an effective strategy to increase productivity while preserving or improving oil quality, with more homogeneous, volumetric heating of microwaves, which minimizes thermal degradation in the aqueous phase compared with prolonged boiling in HD. Some studies showed that HD in the Clevenger-type apparatus typically required long extraction times (90–210 min) to reach yields in the range of 0.35–2.15% (w/w) (Bousbia et al., 2009; Taheri et al., 2023), depending on feed mass, leaf morphology, and process configuration.

In contrast, MAHD reduced extraction time while providing yields comparable to or higher than those under optimized conditions. Elyemni et al. (2021) applied a CCD to MAHD (100 g rosemary, 200–600 W, 20–90 min, water-to-plant ratio 2–6 mL/g, fresh vs. dried material). They stated that power and drying time were the most influential variables, followed by extraction time and water loading. The optimal combination was 600 W, 20 min, a water-to-plant ratio of 2 mL/g, and a drying period of 7 days, resulting in a predicted yield of 1.326% and an experimental maximum of ~1.33%. In comparison to the present study, the extraction time and the solid/liquid ratio were also found to be influential factors. Evaluating the response surface obtained by the author showed that medium-to-high yields were also achieved at 300 W under prolonged extraction times.

Overall, the body of evidence indicates that, for rosemary EO extraction in Clevenger-type systems, conventional HD is robust and capable of high yields but is inherently time- and energy-intensive. MAHD allowed to achieve yields equivalent to or better than the conventional method requiring less time, with lower energy consumption and enhanced oil quality, thanks to consistent levels of oxygenated monoterpenes. These results support scaling up microwave-assisted process may be competitive alternative to conventional HD, particularly when the goal is to maximize both process efficiency and functional quality of rosemary essential oil.

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4. CONCLUSION

RSM combined with desirability functions is an effective strategy for simultaneously optimizing yield and compositional quality of rosemary EO produced by HD and MAHD. For HD, the BBD was applied directly, enabling a detailed quadratic description of the effects and interactions of the four factors on both EO yield and VCI. In contrast, MAHD followed a more economical, hierarchical approach: a screening stage identified the critical variables, which were then studied using a CCC. As a result, the HD models incorporated richer curvature and interaction structure across a broader experimental domain. In contrast, the MAHD models focused on a reduced factor space, achieving robust optimization with fewer experimental runs. However, it is important to note that the MAHD RSM showed lower predictive power compared to the HD RSM. This comparison highlights that HD benefited from a comprehensive multi-factor exploration. At the same time, MAHD was more efficiently optimized by combining screening and RSM, an approach that is advantageous for energy-intensive, non-conventional extraction processes.

For HD, the optimized condition determined by the desirability function was 200 min of extraction, a solid/liquid ratio of 144 g/L, a particle size of 2 mm, and without soaking time. For MAHD, the conditions were a solid/liquid ratio of 80 g/L, 80 min of extraction, and an intermediate microwave power of 350W, achieving competitive yields and VCI contents, while significantly reducing processing time compared with HD. GC-MS analysis showed that both techniques produced qualitatively similar oils, dominated by oxygenated monoterpenes (1,8-cineole, *camphor*, *borneol*, *α*-terpineol). The main challenges in optimizing EO extraction lie in balancing yield maximization with the preservation of thermolabile oxygenated constituents, whose degradation depends on extraction time, temperature, and other process conditions. Moreover, variations related to genotype, cultivation conditions, post-harvest handling, and plant matrix heterogeneity hinder the definition of universal "optimal" parameters, making design-of-experiments and multi-response optimization essential to provide a robust basis for evaluating and defining operating limits for similar matrices. Taken together, these findings support MAHD as a promising, energy-efficient alternative to conventional HD, mainly to produce standardized rosemary EO, where consistent enrichment in bioactive volatiles is required for food, cosmetic, or pharmaceutical applications

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CRediT authorship contribution statement

Mariana C Pedrosa: Conceptualization, Methodology, Investigation, Writing – original draft. Joana S. Amaral: Methodology, Writing – review & editing. Sandrina A. Heleno: Conceptualization, Writing – review & editing. Manuela Pintado: Conceptualization, Project administration, Writing – review & editing. Lillian Barros: Conceptualization & Project administration. Márcio Carocho: Conceptualization, Project administration, Writing – review & editing. All authors reviewed the results and approved the final version of the manuscript.

Conflict of interest

M. Carocho is an editor for Food Chemistry but did not have any involvement in the edition of this manuscript. All other authors have no conflict of interest to disclose.

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This preprint research paper has not been peer reviewed. Electronic copy available at: https://ssrn.com/abstract=6920323

5. REFERENCES

Adams, R. P. (2017). *Identification of essential oil components by gas chromatography/ mass spectrometry* (4.1). Allured publishing.

Aragão, K. K. V., Souza, R. P., Barbosa, S. L. M., da Silva Neta, E. R., Araújo, L. É. P. F., Lopes, A. P., Soares, H. S., Sena, E. P., Azevedo, P. S. da S., de Sousa Neto, B. P., Vasconcelos, D. F. P., Soares, M. J. dos S., de Lima, S. G., Ferreira, P. M. P., & de Oliveira, A. P. (2026). Chemical characterization, toxicity and involvement of calmodulin in the antithrombotic activity of Rosmarinus officinalis L. essential oil. *South African Journal of Botany*, *189*(December 2025), 590–603. https://doi.org/10.1016/j.sajb.2025.12.015

Bousbia, N., Abert Vian, M., Ferhat, M. A., Petitcolas, E., Meklati, B. Y., & Chemat, F. (2009). Comparison of two isolation methods for essential oil from rosemary leaves: Hydrodistillation and microwave hydrodiffusion and gravity. *Food Chemistry*, *114*(1), 355–362. https://doi.org/10.1016/j.foodchem.2008.09.106

Chen, F., Su, X., Yan, T., Fu, X., Wang, Y., Luo, D., & Zhang, Q. (2024). Homogenate-ultrasonic pretreatment followed by microwave hydrodistillation of essential oil from rosemary (Rosmarinus officinalis L.) leaves: Kinetic, chemical composition, and biological activity. *Sustainable Chemistry and Pharmacy*, *42*(August), 101744. https://doi.org/10.1016/j.scp.2024.101744

Conde-Hernández, L. A., Espinosa-Victoria, J. R., Trejo, A., & Guerrero-Beltrán, J. (2017). CO2-supercritical extraction, hydrodistillation and steam distillation of essential oil of rosemary (Rosmarinus officinalis). *Journal of Food Engineering*, *200*, 81–86. https://doi.org/10.1016/j.jfoodeng.2016.12.022

Elyemni, M., Louaste, B., El Ouadrhiri, F., Bouia, A., & Eloutassi, N. (2021). Application of response surface methodology to optimize the extraction of essential oil from Rosmarinus officinalis using microwave-assisted hydrodistillation. *Journal of Applied Pharmaceutical Science*, *11*(1), 129–136. https://doi.org/10.7324/JAPS.2021.110115

Gonzalez-Rivera, J., Campanella, B., Pulidori, E., Bramanti, E., Tiné, M. R., Bernazzani, L., Onor, M., Bàrberi, P., Duce, C., & Ferrari, C. (2023). From volatiles to solid wastes: Towards the full valorization of lavender and rosemary by simultaneous in

Preprint not peer reviewed

This preprint research paper has not been peer reviewed. Electronic copy available at: https://ssrn.com/abstract=6920323

situ microwaves and ultrasounds irradiation extraction. *Industrial Crops and Products*, *194*(June 2022). https://doi.org/10.1016/j.indcrop.2023.116362

Guelifet, K., Kherraz, K., Messaoudi, M., Ferhat, M. A., Khattabi, L., Bendrihem, K. A., Zahnit, W., Addad, D., Benmohamed, M., Azoudj, Y., Harchaoui, L., Aggoun, K., Boumechhour, A., & Rastrelli, L. (2025). Seasonal and Extraction-Dependent Variation in the Composition and Bioactivity of Essential Oils from Wild Rosmarinus officinalis L. *Molecules*, *30*(21), 1–26. https://doi.org/10.3390/molecules30214258

Halimi, M. K. A., Nadaf, M., Riahi Madvar, A., & Rostami, M. (2025). Investigating seasonal variations in composition of the Rosmarinus officinalis essential oil in North Khorasan Province (Bojnord), Iran: a comparative analysis with existing literature, focusing on monoterpenol components. *Natural Product Research*, *0*(0), 1–6. https://doi.org/10.1080/14786419.2025.2565271

Kamli, T. EL, Errachidi, F., Eloutassi, N., Majid, H., Chabir, R., & Bour, A. (2017). Comparaison Quantitative Et Qualitative Des Huiles Essentielles De Rosmarinus Officinalis Obtenues Par Différentes Méthodes. *European Scientific Journal, ESJ*, *13*(21), 172. https://doi.org/10.19044/esj.2017.v13n21p172

Mollova, S., Stanev, S., Dincheva, I., Taneva, I., Mazova, N., Koleva, Y., & Stoyanova, A. (2025). Characteristics of rosemary essential oil obtained by hydrodistillation and steam distillation. *Scientific Study & Research: Chemistry & Chemical Engineering, Biotechnology, Food Industry*, *26*(2), 167–176. https://doi.org/https://doi.org/10.29081/chiba.2025.628

Moradi, S., Fazlali, A., & Hamedi, H. (2018). Microwave-assisted hydro-distillation of essential oil from rosemary: Comparison with traditional distillation. *Avicenna Journal of Medical Biotechnology*, *10*(1), 22–28.

Nieto, G., Ros, G., & Castillo, J. (2018). Antioxidant and Antimicrobial Properties of Rosemary (Rosmarinus officinalis, L.): A Review. *Medicines*, *5*(3), 98. https://doi.org/10.3390/medicines5030098

Rathore, S., Mukhia, S., Kapoor, S., Bhatt, V., Kumar, R., & Kumar, R. (2022). Seasonal variability in essential oil composition and biological activity of Rosmarinus

Preprint not peer reviewed

This preprint research paper has not been peer reviewed. Electronic copy available at: https://ssrn.com/abstract=6920323

officinalis L. accessions in the western Himalaya. *Scientific Reports*, *12*(1), 1–13. https://doi.org/10.1038/s41598-022-07298-x

Sadeh, D., Nitzan, N., Chaimovitsh, D., Shachter, A., Ghanim, M., & Dudai, N. (2019). Interactive effects of genotype, seasonality and extraction method on chemical compositions and yield of essential oil from rosemary (Rosmarinus officinalis L.). *Industrial Crops and Products*, *138*(November 2018), 111419. https://doi.org/10.1016/j.indcrop.2019.05.068

Sakar, E. H., Zeroual, A., Kasrati, A., & Gharby, S. (2023). Combined Effects of Domestication and Extraction Technique on Essential Oil Yield, Chemical Profiling, and Antioxidant and Antimicrobial Activities of Rosemary (Rosmarinus officinalis L.). *Journal of Food Biochemistry*, *2023*, 4–7. https://doi.org/10.1155/2023/6308773

Shahrampour, D., & Razavi, S. M. A. (2023). Fabrication and characterization of novel biodegradable active films based on Eremurus luteus root gum incorporated with nanoemulsions of rosemary essential oil. *Progress in Organic Coatings*, *175*(December 2022), 107360. https://doi.org/10.1016/j.porgcoat.2022.107360

Sprea, R. M., Fernandes, L. H. M., Pires, T. C. S. P., Calhelha, R. C., Rodrigues, P. J., & Amaral, J. S. (2023). Volatile Compounds and Biological Activity of the Essential Oil of Aloysia citrodora Paláu: Comparison of Hydrodistillation and Microwave-Assisted Hydrodistillation. *Molecules*, *28*(11). https://doi.org/10.3390/molecules28114528

Taheri, A., Asadiamirabadi, A., Seyedabadi, M. M., & Kashaninejad, M. (2023). Infrared-assisted hydrodistillation as an efficient, promising approach to the extraction of essential oil from Rosmarinus officinalis. *International Journal of Food Science and Technology*, *58*(4), 2170–2179. https://doi.org/10.1111/ijfs.16293

Teshale, F., Narendiran, K., Beyan, S. M., & Srinivasan, N. R. (2022). Extraction of essential oil from rosemary leaves: optimization by response surface methodology and mathematical modeling. *Applied Food Research*, *2*(2). https://doi.org/10.1016/j.afres.2022.100133

Vital, A. C. P., Guerrero, A., Monteschio, J. D. O., Valero, M. V., Carvalho, C. B., De Abreu Filho, B. A., Madrona, G. S., & Do Prado, I. N. (2016). Effect of edible and

Preprint not peer reviewed

This preprint research paper has not been peer reviewed. Electronic copy available at: https://ssrn.com/abstract=6920323

active coating (with rosemary and oregano essential oils) on beef characteristics and consumer acceptability. *PLoS ONE*, *11*(8), 1–15. https://doi.org/10.1371/journal.pone.0160535

Walid, Y., Majdi, H., Wissem, A. W., Khammassi, S., Mahmoud, G., Manef, A., Ksouri, R., & Saidani Tounsi, M. (2019). Deterpenation of rosemary (Rosmarinus officinalis var. typicus L.) leaf essential oil using a mathematical model of kinetic guided hydrodistillation. *Journal of Essential Oil Research*, *31*(3), 235–246. https://doi.org/10.1080/10412905.2018.1562387

Xia, N., Wang, J., Jia, Y., Duan, J., Wang, X., Li, J., Zhou, P., Xie, Y., Shi, H., Zhao, C., Zou, J., Guo, D., Shi, Y., Li, H., Wu, Z., Yang, M., Chang, X., Sun, J., & Zhang, X. (2025). Optimization of the process of extracting essential oil of rosemary by hydro distillation with different auxiliary methods. *Lwt*, *215*(October 2024), 117266. https://doi.org/10.1016/j.lwt.2024.117266

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This preprint research paper has not been peer reviewed. Electronic copy available at: https://ssrn.com/abstract=6920323