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Optimizing Thermal Pretreatment for Volatile Bioactive Profiling in Medicinal Plants Using HS-GC-MS Analysis

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Abstract

Oregano (*Origanum vulgare* L.), basil (*Ocimum basilicum* L.), rosemary (*Rosmarinus officinalis* L.), yarrow (*Achillea millefolium* L.), and thyme (*Thymus vulgaris* L.) are aromatic medicinal plants rich in bioactive volatile compounds with antioxidant, antimicrobial, and anti-inflammatory properties. This study presents a simple, solvent-free, and eco-friendly headspace GC-MS method for VOC profiling. Optimized thermal pretreatment (40–90 °C) enhanced compound detection, particularly at 70–90 °C, without loss of reproducibility. The approach lowers analytical costs and waste generation, supporting green analytical practices and the sustainable valorization of medicinal herbs as natural functional ingredients.

Keywords: volatile plant-based bioactive compounds; sustainable sample preparation; headspace GC-MS; green analytical methodology; valorization of medicinal plants

1. Introduction

Medicinal plants and spices represent a rich and underutilized source of plant-based bioactive compounds, including volatile organic compounds (VOCs), which are increasingly sought after for their potential health benefits and functional applications [1,2]. These natural substances—primarily terpenoids, benzene derivatives, and phenylpropanoids—exhibit well-documented antioxidant, anti-inflammatory, antimicrobial, and metabolic regulatory activities, making them valuable ingredients in the development of nutraceuticals, functional foods, and natural therapeutics. In this context, the valorization of VOC-rich medicinal plants aligns with global efforts toward sustainable sourcing and the circular use of botanical resources.

The crucial relevance of medicinal plants and spices might be explained not only by their definite and unique organoleptic properties, but also their pronounced health-promoting impact [1–4]. Therefore, the implications of several medicinal plants are increasingly at the forefront of interest, along with the increasing amount of research data published on their positive biological impact [1–8]. As they are widely used in the food industry and natural medicine, reliable analysis and accurate detection are of utmost significance. Hence, our study focuses on the elaboration of an efficient analytical method based on an improved sample-preparation procedure with improved environmentally friendly features. The focus point of our research was to investigate the association between

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variations in thermal treatment conditions and the detectability of volatile components in five distinctive medicinal plant species.

Among the most promising species are oregano (*Origanum vulgare* L.), basil (*Ocimum basilicum* L.), rosemary (*Rosmarinus officinalis* L.), thyme (*Thymus vulgaris* L.), and yarrow (*Achillea millefolium* L.), all of which possess diverse and potent volatile profiles (Table 1). Key bioactive constituents such as thymol, carvacrol, eucalyptol (1,8-cineole), limonene, and eugenol are known to contribute to both plant defense and beneficial physiological effects in animals and humans. Their inclusion in animal feed, for instance, can enhance digestion, modulate the gut microbiota, reduce inflammation, and support immune function—making them attractive natural alternatives to synthetic additives or antibiotics. From a human health perspective, these phytochemicals may support cardiovascular health, cognitive function, stress reduction, and cellular protection against oxidative stress [3–8].

Table 1. The main VOCs in tested medicinal plants.

Medicinal Plants	Monoterpenes	Sesquiterpenes	Oxygenated Compounds	Benzene Derivatives
Oregano (*Origanum vulgare* L.)	carvacrol	β-caryophyllene	linalool	
	thymol	germacrene D	terpinen-4-ol	
	p-cymene			
	γ-terpinene			
Basil (*Ocimum basilicum* L.)	α-pinene	eugenol	linalool	estragole
	β-pinene	β-caryophyllene		methyleugenol
	1,8-cineole	germacrene D		
Rosemary (*Rosmarinus officinalis* L.)	α-pinene	β-caryophyllene	verbenone	
	1,8-cineole	α-humulene	thujone	
	camphor			
	borneol			
Thyme (*Thymus vulgaris* L.)	thymol	β-caryophyllene	linalool	
	carvacrol	germacrene D	terpinen-4-ol	
	γ-terpinene			
	p-cymene			
Yarrow (*Achillea millefolium* L.)	camphor	chamazulene	borneol	
	1,8-cineole	β-caryophyllene	thujone	
	sabinene	germacrene D		
	α-pinene			

Furthermore, terpenoids, such as thymol, carvacrol, borneol, eucalyptol, camphene, caryophyllene, limonene, and benzene derivatives (e.g., estragole, eugenol) have antioxidant [3,4], anti-inflammatory [5], antibacterial, and antifungal properties [6–8]. Another report suggested that limonene, borneol, geranial, and other terpenoids might have antidiabetic and antihypertensive properties [9]. The abovementioned natural, volatile compounds constitute an integral part of the defense mechanism of plants, while exerting positive effects on animal health and performance; moreover, by entering the food chain, they are also particularly important in human nutrition (SDG 3) [10,11]. The addition of medicinal plants and spices to the feed of dairy cattle may have a positive contribution to animal health and welfare, as well as milk production [12,13].

The studied volatile compounds have antimicrobial properties that can help to reduce the levels of pathogenic bacteria in the intestinal tract. This can improve the digestion processes and contribute to better feed utilization [14].

An additional physiological advantage of the volatile compounds of the investigated medicinal plants is that their anti-inflammatory effect can help to minimize intestinal inflammation, which might be a common problem for animals, especially in intensive feeding systems [15].

It is essential that volatile components, e.g., terpenes, support the immune system of animals via their antioxidant properties, which is particularly important for farm animals kept under stressful conditions [16].

Terpenes, deriving from medicinal plants rich in volatile compounds, might be considered as natural alternatives to traditional antibiotics and growth promoters, whose use is increasingly regulated or restricted in animal husbandry [17]. Thus, these medicinal and aromatic plants can be important as alternatives to conventional feed supplements in achieving SDG targets.

Naturally, the abovementioned properties of the selected plants can be observed not only in respect of animal welfare, but also with relevance in human health [1,2,5,17–19]. Therefore, their regular consumption and integration into the human diet are of particular importance for contemporary societies that are exposed to increased stress factors and civilization-related harms. Each of the studied plants contributes to maintaining health and preventing various diseases [1,16–18].

On the other hand, the natural antioxidants they contain provide protection against cellular damage and can slow down aging processes [17]. Due to their effective anti-inflammatory and antimicrobial properties, these medicinal plants can help mitigate harmful processes occurring in the human body [18].

Oregano, rosemary, and basil, through their inherent antioxidant compounds, help to neutralize the harmful effects of free radicals that are continuously generated in the body [19]. These plants stimulate the production of digestive enzymes, promote the efficient absorption and bioutilization of nutrients, and reduce bloating or digestion disorders [1]. The antibacterial, antiviral, and antifungal properties of these medicinal plants contribute to preventing infections and strengthen the immune system, making the body more resistant to diseases. The essential oils of rosemary and basil may improve memory, reduce stress, and support the health of the nervous system [20–23]. This is particularly significant in today's stressful lifestyle.

However, despite the growing interest in these natural volatiles, their analytical detection remains a challenge due to the instability and low abundance of many VOCs. Traditional extraction and purification methods typically involve labor-intensive protocols, high solvent consumption, and expensive instrumentation, which run counter to green chemistry principles. Most of the applied methods require the application of very extensive sample-preparation steps prior to the analysis of the extracted essential oils, mostly via gas chromatography combined with mass spectrometry (GC-MS) [24–28]. Traditional and widely used sample-preparation procedures require complex and expensive devices (e.g., cryogenic mill, sonic equipment, centrifuge solid phase microextraction, rotary evaporator, etc.), and in many cases, large amounts of organic solvents, in addition to the production of considerable amounts of waste [25–31].

To address these limitations, our study proposes a simplified and environmentally friendly analytical approach for VOC profiling using headspace gas chromatography–mass spectrometry (HS-GC-MS).

The core objective of our research was to investigate how different thermal pretreatment conditions affect the composition and detectability of volatile constituents in the

selected medicinal plants. As VOCs are highly sensitive to temperature and prone to degradation, careful optimization of sample preheating is essential for accurate and reproducible analysis. Our enhanced method minimizes organic solvent use, reduces sample-preparation time, and eliminates the need for complex equipment—thereby supporting the development of sustainable volatilomic workflows.

This work contributes to the valorization of VOC-rich medicinal plants by improving access to their bioactive profiles through greener analytical practices. The findings provide a foundation for future applications of these plants in sustainable health-promoting products, including functional foods, nutraceuticals, and natural preservatives.

2. Materials and Methods

2.1. Raw Materials

All the examined medicinal plants were purchased from Fitodry Ltd. (Tiszaföldvár, Hungary). Herb samples were analyzed using near-infrared reflectance spectroscopy (NIRS) by Cumberland Valley Analytical Services (CVAS, Waynesboro, PA, USA), with calibrations based on wet chemistry reference datasets (Table 2).

Table 2. Nutrient values of the medicinal plants during the treatment (g/kg of DM).

	Oregano	Basil	Thyme	Rosemary	Yarrow
dry matter	931.0	884.0	911.0	925.0	902.0
crude protein	107.0	248.0	169.0	143.0	104.0
neutral detergent fiber	309.0	384.0	381.0	288.0	522.0
lignin	82.4	69.8	75.9	48.7	106.0

2.2. Sample Preparation

In headspace HS-GC-MS analysis, thermal pretreatment of the samples plays a critical role in the detectability of VOCs [32,33]. The effect of temperature is significant in several ways, as its increase facilitates the release of volatile components [32–34]. This enhances the detectable concentration during the gas chromatographic (GC) injection, resulting in better sensitivity and detectability.

Moreover, increasing the temperature accelerates the attainment of equilibrium. However, at excessively high temperatures, heat-sensitive compounds may degrade or undesirable reactions (e.g., oxidation, Maillard reactions) may occur. This can distort the true VOC profile or lead to the loss of important components [34–36].

Matrix effects, such as the type of sample (e.g., plant tissue, powder, extract), also influence how the sample responds to heat treatment. For instance, in plant matrices, heat may rupture cell walls, releasing additional VOCs that otherwise would remain undetected.

Therefore, the establishment of the optimal temperature is crucial. Thus, to avoid unnecessary analytical interference, all the steps of the pretreatment were chosen carefully according to the properties of the targeted molecules (Table 3).

Based on the assessment of the data in Table 3, the dried and finely grounded (<100 µm) samples were measured after a gentle preheating process. No complicated time- and reagent-consuming sample pretreatments were used, as the samples were pretreated only mechanically by grounding. The plant material was sheltered from direct sunlight and then stored in double-layered, aroma-sealed aluminum bags at temperatures of 3–5 °C until processing.

In the GC-MS measurement, the preheating temperatures varied between 40 °C and 90 °C as a temperature optimization program. Because most of the tested components remain stable at temperatures up to 90 °C, if higher temperatures are used (e.g., 110–130 °C), the determination of stable components may be better, but the risk of oxidation and

degradation increases significantly [33,34]. Since our goal was to detect the highest possible number of volatile components, a temperature optimization experiment was performed at different temperatures to determine the most optimal pretreatment temperature. The applied temperature range during the pretreatment is crucial because volatile organic compounds such as terpenes and their derivatives in the studied plant samples can be present in the analytical gas space above the sample even at fairly low temperatures [32–36].

Table 3. Boiling point and thermal stability of the main VOCs in tested medicinal plants.

Components	Boiling Point (°C)	Thermal Stability
α-pinene	156	Moderately stable, may be oxidized
β-pinene	164	Moderately stable, may be oxidized
limonene	176	Stable, but can be oxidized
β-caryophyllene	262	Stable
1,8-cineole (eucalyptol)	176	Stable
linalool	198	Moderately stable, may be oxidized
methyl chavicol (estragol)	216	Stable
eugenol	251	Stable
thymol	233	Stable
carvacrol	237	Stable
camphor	204	Stable
chamazulene	240	Stable, but photosensitive
α-bisabolol	153	Moderately stable
borneol	210	Stable
verbenone	227	Stable
α-terpineol	217	Stable
γ-terpinene	183	Moderately stable, may be oxidized
p-cymene	177	Stable
geraniol	230	Moderately stable, may be oxidized
sabinene	163	Moderately stable, may be oxidized
camphene	160	Moderately stable
α-humulene	275	Stable
fenchone	193	Stable
thujol	200	Stable
sabinol	224	Stable
α-cubebene	260	Stable
isoborneol	212	Stable
methyleugenol	254	Stable
bornyl acetate	229	Stable
myrcene	166	Moderately stable, may be oxidized
terpinen-4-ol	209	Stable
neryl acetate	250	Stable

2.3. GC-MS Analysis

To test the detectability and thermal stability of volatile components, measurement methods and descriptions were prepared based on an ascending heat profile and temperature preparation.

The metabolome and volatilome were profiled by gas chromatography (8890 GC, Agilent Technologies, Santa Clara, CA, USA) with an Agilent 7000D GC/TQ mass-selective detector. The mass spectrometer was tuned using perfluorotributylamine with masses *m/z* 69.0, 264.0, and 502.0.

The Gerstel MAESTRO 1.4 software platform was used for efficient automated sample preparation and introduction. The GC-MS system was operated by Agilent MassHunter Acquisition 10.0.384.1 software. To evaluate the obtained data, Mass Spectral Library (NIST 17) was used.

The measurement required the development of 3 methods:

1. Gerstel MPS method

2. GC method
3. MS method

In the MPS Headspace Gerstel method, a 2500 µL 65 mm HS syringe was used at 90 °C with 60 s flush time. For sample preparation, the incubation temperature of the agitator ranged from 40 to 90 °C according to the applied gentle temperature optimization to avoid decomposing processes [32–36]. The incubation time was 5.00 min and the agitator speed was 250 rpm. In setting the incubation time, it was very important to use a relatively short time because if the sample is incubated for a longer time (e.g., 20–30 min), a conversion of oxidation-prone compounds (e.g., pinene, terpinene, linalool) may take place.

The injected total volume was 2000.0 µL with 200.00 µL/s injection speed. For the injection, split mode was used with a 10:1 split ratio, so the injected sample volume was 200 µL. In the inlet, the heater temperature was 250 °C, with a total flow of 6.4259 mL/min, and the septum purge flow was 5 mL/min.

In the GC method, the sample inlet was GC with automatic injection. The method run time was 30.333 min, without post-run time.

To perform volatilomic analysis, the temperature program was as follows:

The GC column was operated in the temperature-programmed mode with an initial oven temperature of 40 °C (held for 1 min) and ramp at 180 °C with a 5 °C min^{-1} rate, with 0 min holding time. To eliminate residuals and avoid carryover, a heating-up ramp was used at 300 °C with a 90 °C min^{-1} rate and 0 min holding time.

An Agilent 19091S-433UI: 0378217H HP-5MS UI column with 30 m × 250 µm × 0.25 µm dimensions was used. The inert gas was helium (99.999%), flow rate was 0.475 mL/min, average velocity was 25 cm/s, and holdup time was 2 min. The MSD transfer line temperature was 325 °C.

In the MS method, the ion source was EI (70 eV), the source temperature was 230 °C, and a 1 min solvent delay was applied. The data was carried out over a mass range of *m/z* from 20 to 500; the step size was 0.1 *m/z* in scan mode.

The HS-GC-MS analysis of organic compounds from the examined herbal samples was performed using 20 mL headspace vials containing 2 g of the dried and ground material. The vials were sealed with PTFE-lined septum and an aluminum crimp cap, and then conditioned for 20 min at 90 °C.

3. Results

In our study, the sample-preparation temperature (agitator temp.) was increased from 40 °C by steps of 10 °C up to 90 °C. We refrained from assessing temperatures higher than 90 °C to avoid the potential degradation of certain volatile analytes [37]. At each temperature, the chromatograms of the different plant samples (basil, yarrow, thyme, oregano, and rosemary) were recorded (Figures S1–S5; see Supplementary S1–S7). The chromatograms obtained for each plant species at different sample-pretreatment temperatures are presented in Supplementary S1–S7. In addition to the chromatograms, the names of the identified compounds (based on PubChem), the PubChem IDs, and the CAS numbers are presented, as well as the retention times (Rts) and the base of their identification (matching in the spectral library data) in separated tables (Tables 4–9). The identification of a compound was accepted when the match with the spectral library data exceeded 95%. Compound identification in this study was performed tentatively based on comparison of the obtained mass spectra with the NIST library. While spectral matching provides strong evidence for the presence of the reported compounds, retention indices could not be determined due to the lack of an normal-alkane calibration series. Therefore, all compound identifications should be regarded as putative. It should be noted that the primary aim of this study was not the precise identification of individual compounds, but rather to investigate how

thermal sample pretreatment affects the appearance of components in the headspace, an approach consistent with common practice in GC–MS studies of complex mixtures.

Table 4. Compounds in basil (*Ocimum basilicum* L.) identified by the HS-GC-MS method.

			Retention Time (Rt) of Identified Compounds at Different Agitator Temperatures (°C)					
Compound Name (PubChem)	**PubChem CID**	**CAS**	**40 °C**	**50 °C**	**60 °C**	**70 °C**	**80 °C**	**90 °C**
(1R)-camphor	159055	464-49-3				16.5014	16.4858	16.4818
(+)-3-carene	443156	498-15-7					15.024	
β-bourbonene	62566	5208-59-3					23.2707	23.2732
(+)-α-pinene	82227	7785-70-8					9.9716	9.9727
β-pinene	49868219	18252-44-3					25.7312	
α-phellandrene	7460	99-83-2				9.7605		12.1298
α-pinene	6654	80-56-8			9.9783	9.9823		
α-terpineol	17100	98-55-5					17.7947	17.7972
myrcene	31253	123-35-3				11.6963	11.6853	11.6889
β-ocimene	18756	13877-91-3					13.4478	
γ-muurolene	12313020	30021-74-0					26.5062	
γ-terpinene	7461	99-85-4					13.8103	13.8114
cis-β-ocimene	5320250	3338-55-4						13.4508
α-terpinene	7462	99-86-5					12.5116	12.5159
linalool formate	61040	115-99-1						15.0389
cis-linalool oxide	6428573	5989-33-3						14.2478
trans-2-hexenal	5281168	505-57-7						7.6062
trans-2-hexenal	5281168	6728-26-3						7.6064
thymol	78153	4427-56-9				20.5406		
methyl cinnamate	637520	103-26-4			23.0977	23.1056	21.0057	21.0083
methyl cinnamate	637520	1754-62-7		23.1025		21.0081		21.0082
3-carene	26049	13466-78-9		15.0242	15.0242	15.0212	13.4475	12.3174
terpinene-4-yl-acetate	20960	4821-04-9					17.4198	17.4208
sabinene	86182191	16626-39-4						9.5193
bornyl acetate	6448	92618-89-8					20.5162	20.5156
benzaldehyde	240	100-52-7				10.7862	10.782	10.7805
phenylacetaldehyde	998	122-78-1					13.3497	13.3509
(-)-camphene	440966	5794-04-7				10.4421	10.4356	10.433
α-thujene	17868	2867-05-2				11.1906	9.7592	9.7544
sabinene	18818	3387-41-5						11.1905
(-)-β-pinene	440967	18172-67-3			11.3063	11.3081	11.2974	11.3017
3-methylbutanal	11552	590-86-3						3.6033
camphene	6616	79-92-5				10.4411		
(-)-caryophyllene	5281515	87-44-5					24.1898	
copaene	12303902	3856-25-5						23.0054
β-elemene	6918391	515-13-9					23.3915	
limonene	68140	499-97-8					11.1891	
menthone	26447	89-80-5						16.7341
(+)-limonene	440917	5989-27-5				12.8885		
estragole	8815	140-67-0	17.9963	18.0069	18.0024	18.0002	18.0074	18.0223
1,8-cineole	2758	470-82-6	12.9959	12.9961	12.9895	12.9823	12.9824	
fenchol	15406	1632-73-1					15.5101	15.5145
(±)-fenchone	14525	1195-79-5					14.7593	14.7555
bornyl acetate	107217	13851-11-1					18.6525	18.6551
2-ethylfuran	18554	3208-16-0						4.1854
2-methylheptanal	86044	16630-91-4						3.6053
hexanal	6184	66-25-1				6.2289		6.2036
humulene	5281520	6753-98-6					25.054	

Table 4. *Cont.*

			Retention Time (Rt) of Identified Compounds at Different Agitator Temperatures (°C)					
Compound Name (PubChem)	**PubChem CID**	**CAS**	**40 °C**	**50 °C**	**60 °C**	**70 °C**	**80 °C**	**90 °C**
isobornyl formate	23623868	1200-67-5					17.1005	17.1007
(-)-fenchone	82229	7787-20-4					14.7591	
linalyl acetate	8294	115-95-7						15.0389
methyleugenol	7127	93-15-2				23.5814	23.582	
thymol	6989	89-83-8				20.5398		
α-*cis*-bergamotene	6429303	18252-46-5				24.4837	24.4807	
p-cymene	7463	99-87-6		12.7593	12.7561	12.7585	12.7552	12.7636

Note: All compounds were identified according to their retention time and mass spectrometry data.

Table 5. Compounds in yarrow (*Achillea millefolium* L.) identified by the HS-GC-MS method.

			Retention Time (Rt) of Identified Compounds at Different Agitator Temperatures (°C)					
Compound Name (PubChem)	**PubChem CID**	**CAS**	**40 °C**	**50 °C**	**60 °C**	**70 °C**	**80 °C**	**90 °C**
(1R)-camphor	159055	464-49-3			16.4993	16.4965	16.4889	16.481
(+)-3-carene	443156	498-15-7					15.0219	15.0211
(-)-carvone	439570	6485-40-1					19.3456	19.3415
(+)-α-pinene	82227	7785-70-8					9.979	9.9787
α-phellandrene	7460	99-83-2				9.7653	12.1382	12.063
α-pinene	6654	80-56-8			9.9817	9.9785		
α-terpineol	17100	98-55-5				17.7999	17.7986	17.7952
myrcene	31253	123-35-3				11.6942	11.6932	11.6908
β-ocimene	18756	13877-91-3					11.8603	
β-pinene	14896	127-91-3		11.3049				
γ-terpinene	7461	99-85-4				14.0845	13.8188	13.8134
myrcene	564723	29548-02-5						16.8134
β-ocimene	527280	42123-66-0				11.9374	11.9359	11.9321
cis-β-ocimene	5320250	3338-55-4					11.8604	11.8593
α-terpinene	7462	99-86-5				12.5177	12.5186	12.5152
artemisia ketone	68346	546-49-6				13.8596	13.8569	13.8552
1-nonene	31285	124-11-8				8.6507	8.6577	
1-(2-methyloxolan-2-yl)ethan-1-one	538312	32318-87-9		30.0926			15.1577	
piperityl acetate	102578	1204-30-4						17.421
3-(4-methylbenzoyl)-2-thioxo-1,3-thiazol-4-yl-4-methylbenzoate	576784	299929-13-8				19.0661		
3-carene	26049	13466-78-9				15.0231	9.6249	11.859
lavandulyl acetate	30247	25905-14-0					20.5301	
terpinene-4-yl-acetate	20960	4821-04-9					17.4244	17.4211
anethole	637563	104-46-1		18.0022				
trans-anethole	637563	4180-23-8		18.0018				
benzaldehyde	240	100-52-7				10.783	10.7854	10.7857
phenylacetaldehyde	998	122-78-1						13.351
(-)-camphene	440966	5794-04-7				10.4435	10.4433	
α-thujene	17868	2867-05-2		11.1953	9.7634		9.7635	9.7603
β-thujene	524198	36262-09-6						10.6046
β-thujone	91456	471-15-8						15.2895
sabinene	18818	3387-41-5		11.192	11.198	11.1953	11.2016	
(-)-*trans*-pinocarveol	1201530	547-61-5						16.2965
(-)-β-pinene	440967	18172-67-3		11.3042	11.3058	11.3037	11.3115	

Table 5. *Cont.*

Compound Name (PubChem)	PubChem CID	CAS	Retention Time (Rt) of Identified Compounds at Different Agitator Temperatures (°C)					
			40 °C	50 °C	60 °C	70 °C	80 °C	90 °C
camphene	6616	79-92-5						10.4402
carveol	7438	99-48-9					19.0619	
carvone	7439	99-49-0					19.3493	
limonene	68140	499-97-8				11.1952	11.2016	11.2082
terpinolene	11463	586-62-9				14.7353	14.734	
sylvestrene	12304570	1461-27-4					12.8954	
isoterpinolene	102443	586-63-0						14.7307
1-cyclopropyloctane	524687	1472-09-9					8.6573	
d-carvone	16724	2244-16-8						19.3415
(+)-limonene	440917	5989-27-5				12.8977	12.8961	12.8981
estragole	8815	140-67-0	18.0039		18.0062	18.0012	18.0017	18.003
1,8-cineole	2758	470-82-6		13.0054	12.9982	12.9838	12.9812	12.9797
hexanal	6184	66-25-1				6.2232	6.2163	6.2042
isoborneol	6321405	124-76-5					17.0961	17.0933
myrtenyl acetate	11435490	1079-01-2						16.2961
santolina triene	519872	2153-66-4				9.164	9.1577	9.1546
α-thujone	261491	546-80-5					15.2915	
tricosane	12534	638-67-5				30.1258		
cyclofenchene	79022	488-97-1						9.6222
p-cymene	7463	99-87-6		12.7616	12.7589	12.7585	12.759	12.7573
(E)-β-ocimene	5281553	3779-61-1		15.0251			13.1268	
(-)-caryophyllene	5281515	87-44-5				20.5295		

Note: All compounds were identified according to their retention time and mass spectrometry data.

Table 6. Effects of the pretreatment temperature on the peak area at chosen compounds.

	Temperature (°C)					
	40	50	60	70	80	90
1,8-cineole in thyme	421,443	962,724	1,609,215	4,346,724	35,903,383	81,501,286
p-cymene in rosemary	1,117,650	1,517,120	5,050,374	61,474,306	284,797,019	340,913,865
D-limonene in oregano	503,005	1,101,064	1,785,263	3,399,726	11,838,252	32,282,742

Table 7. Compounds in thyme (*Thymus vulgaris* L.) identified by the HS-GC-MS method.

Compound Name (PubChem)	PubChem CID	CAS	Retention Time (Rt) of Identified Compounds at Different Agitator Temperatures (°C)					
			40 °C	50 °C	60 °C	70 °C	80 °C	90 °C
(1R)-camphor	159055	464-49-3				16.4935	16.4884	16.4855
(1S,3R,6R)-(-)-4-carene	530422	29050-33-7					12.5217	12.5381
(+)-α-pinene	82227	7785-70-8				9.9703	9.9759	
α-phellandrene	7460	99-83-2		9.762		9.7575	12.1341	12.142
α-pinene	6654	80-56-8	9.9721	9.9788	9.9782	9.9702		
α-terpineol	17100	98-55-5						17.7948
myrcene	31253	123-35-3	11.6817	11.6921	11.6922	11.6847	11.6908	11.7008
β-ocimene	18756	13877-91-3						13.4604
β-phellandrene	11142	555-10-2						11.1954
β-pinene	14896	127-91-3			15.0207			
γ-terpinene	7461	99-85-4	13.8054	13.8144	13.8116	13.8062	13.8176	13.8373
chamazulene	576718	3479-89-8						11.1091
linalool formate	61040	115-99-1		15.0249	15.0208		15.0181	15.0167
methyl-3-methyl-2-butenoate	13546	924-50-5						7.3644

Table 7. *Cont.*

			Retention Time (Rt) of Identified Compounds at Different Agitator Temperatures (°C)					
Compound Name (PubChem)	**PubChem CID**	**CAS**	**40 °C**	**50 °C**	**60 °C**	**70 °C**	**80 °C**	**90 °C**
thymol	78153	4427-56-9	20.5386	20.5469	20.5506	20.5459	20.5493	20.5447
methyl cinnamate	637520	1754-62-7				23.0994		
3-methylbut-3-en-1-ol	12988	763-32-6					4.7362	4.7342
3-carene	26049	13466-78-9					12.3235	9.9891
5-methylhexan-3-one	12187	623-56-3						8.5449
carvacrol	72855	3228-03-3					20.8264	
3-octanol	11527	589-98-0					11.8105	11.8183
3-octanone	246728	106-68-3					11.5503	11.5543
terpinen-4-yl-acetate	20960	4821-04-9					17.4208	
sabinene	86182191	16626-39-4						9.5282
acetic acid	176	64-19-7					2.9092	2.8706
trans-anethole	637563	4180-23-8						17.9968
isobutylbenzene	10870	538-93-2						10.3092
1-methoxy-2-(propan-2-yl)-4-methylbenzene	161716	31574-44-4					19.0083	19.0057
2-m-tolylpropene	70759	1124-20-5					14.7456	14.7462
carvacrol methyl ether	80790	6379-73-3				19.2714		
thymyl methyl ether	14104	1076-56-8			19.0053	19.0033		
methyl benzoate	7150	93-58-3					14.9215	14.9241
(-)-camphene	440966	5794-04-7	10.4309	10.4414	10.4408	10.436		
α-thujene	17868	2867-05-2	9.7511	9.7618	9.7647	9.7581	9.7576	9.7649
β-thujene	520384	28634-89-1					11.1914	11.1943
sabinene hydrate	62367	208-911-7					17.4208	17.419
sabinene	18818	3387-41-5					11.1933	
(-)-β-pinene	440967	18172-67-3				15.0176		
β-caryophyllene	564746	242794-76-9				24.1842		
2-methylbutanal	7284	96-17-3						3.6207
3-methylbutanal	11552	590-86-3						3.6204
methyl-2-methylbutyrate	13357	868-57-5				5.7001	5.6761	5.6915
4-pentenyl-butyrate	520485	30563-31-6					13.9852	13.9842
camphene	6616	79-92-5			10.4411	10.4363	10.436	10.4443
(-)-caryophyllene	5281515	87-44-5					24.1916	24.1891
limonene	68140	499-97-8						11.3052
terpinolene	11463	586-62-9		12.5171	12.5139	12.5093		
(+)-limonene	440917	5989-27-5		12.8889	12.8886	12.8841		
dimethyl ether	8254	115-10-6					10.4577	
estragole	8815	140-67-0	17.9938	18.0048	18.0029	17.9965	17.9977	17.9979
1,8-cineole	2758	470-82-6	12.9998	13.0046	12.9998	12.9868	13.0048	13.054
isoborneol	6321405	124-76-5				17.0934		17.0925
limonene	22311	138-86-3					12.9222	12.9863
carvacrol	10364	499-75-2						20.8223
tetracosane	12592	646-31-1					30.1598	
thymol	6989	89-83-8	20.5385					
toluene	1140	108-88-3					5.4656	5.4649
cyclofenchene	79022	488-97-1			9.9782		9.6168	12.3307
tricyclene	79035	508-32-7						9.6219
3-hexenyl-isobutanoate	5352539	41519-23-7						16.315
borneol	64685	507-70-0				17.0933	17.0951	17.0926
o-cymene	10703	527-84-4					12.861	
4-propenylphenol	5314058	1000429-54				14.7365		
p-cymene	7463	99-87-6	12.7468	12.7553	12.756	12.7527	12.7718	

Table 8. Compounds in oregano (*Origanum vulgare* L.) identified by the HS-GC-MS method.

			Retention Time (Rt) of Identified Compounds at Different Agitator Temperatures (°C)					
Compound Name (PubChem)	**PubChem CID**	**CAS**	**40 °C**	**50 °C**	**60 °C**	**70 °C**	**80 °C**	**90 °C**
(1R)-camphor	159055	464-49-3				16.4983	16.5012	16.4939
(+)-3-Carene	443156	498-15-7					19.5625	15.0236
(1S,3R,6R)-(-)-4-carene	530422	29050-33-7						12.5144
(+)-α-pinene	82227	7785-70-8					9.9778	9.9781
α-phellandrene	7460	99-83-2					12.1363	12.1357
α-pinene	6654	80-56-8	9.9778	9.9817	9.9804	9.9784		
α-terpineol	17100	98-55-5						17.7992
β-bisabolene	10104370	495-61-4				26.2744	26.2762	
myrcene	31253	123-35-3				11.6927	11.6909	11.6915
β-pinene	14896	127-91-3					11.3101	11.3037
γ-terpinene	7461	99-85-4				13.8139	13.8144	13.8165
cis-β-ocimene	5320250	3338-55-4					13.452	13.4553
α-terpinene	7462	99-86-5					12.5138	12.5142
2,3-hexanedione	19707	3848-24-6					6.081	
trans-2-hexenal	5281168	505-57-7					7.6217	
trans-2-hexenal	5281168	6728-26-3					7.6188	7.601
2-isopropyl-4-methylphenol	78153	4427-56-9	20.8162	20.8242	20.8299	20.5458	20.5479	20.5493
3-carene	26049	13466-78-9			15.0256	15.0228	12.3226	12.3206
2,4-dimethyl-3-pentanone	11271	565-80-0					6.0806	6.0595
terpinene-4-yl-acetate	20960	4821-04-9					17.425	17.4241
6-methyl-5-hepten-2-one	9862	110-93-0						11.5743
anethole	637563	104-46-1	17.995					
benzaldehyde	240	100-52-7					10.7868	10.7864
1-methoxy-2-(propan-2-yl)-4-methylbenzene	161716	31574-44-4					19.2819	
carvacrol methyl ether	80790	6379-73-3						19.2821
thymyl methyl ether	14104	1076-56-8						19.0097
(-)-camphene	440966	5794-04-7					10.4427	10.4383
α-thujene	17868	2867-05-2	9.9778				9.7656	9.7632
1-isopropyl-4-methylenebicyclo[3.1.0]hex-2-ene	524198	36262-09-6						10.6103
3-thujanone	11027	1125-12-8					15.2874	
(-)-β-pinene	440967	18172-67-3				11.3086	11.3104	11.3038
methyl-2-methylbutyrate	13357	868-57-5					5.7127	5.7031
camphene	6616	79-92-5				10.4462	10.4428	10.4385
(-)-caryophyllene	5281515	87-44-5					24.1928	
limonene	68140	499-97-8						19.5635
terpinolene	11463	586-62-9					12.5135	
isoterpinolene	102443	586-63-0					14.7338	
β-terpinene	66841	99-84-3						11.1964
(+)-limonene	440917	5989-27-5	12.8823	12.8906	12.8906	12.889	12.8903	12.8947
estragole	8815	140-67-0		18.0079	18.0032	18.0026	18.0012	18.0003
1,8-cineole	2758	470-82-6	12.9929	12.995	12.9899	12.9842	12.9818	12.9841
isoborneol	6321405	124-76-5				17.1014	17.0999	17.0979
carvacrol	10364	499-75-2						20.8321
isobutyl isobutyrate	7351	97-85-8						9.3615
α-thujone	261491	546-80-5						15.2864
thymoquinone	10281	490-91-5						19.4897
cyclofenchene	79022	488-97-1						9.6182
α-*cis*-bergamotene	6429303	18252-46-5					24.4841	

Table 8. *Cont.*

Compound Name (PubChem)	PubChem CID	CAS	Retention Time (Rt) of Identified Compounds at Different Agitator Temperatures (°C)					
			40 °C	50 °C	60 °C	70 °C	80 °C	90 °C
borneol	64685	507-70-0				17.1009		17.0978
p-cymene	7463	99-87-6	12.7542	12.7611	12.7609	12.7578	12.7583	12.7643
(E)-β-ocimene	5281553	3779-61-1						13.1289

Note: All compounds were identified according to their retention time and mass spectrometry data.

Table 9. Compounds in rosemary (*Rosmarinus officinalis* L.) identified by the HS-GC-MS method.

Compound Name (PubChem)	PubChem CID	CAS	Retention Time (Rt) of Identified Compounds at Different Agitator Temperatures (°C)					
			40 °C	50 °C	60 °C	70 °C	80 °C	90 °C
α-pinene	6654	80-56-8	9.9762	9.9811				
(-)-camphene	440966	5794-04-7	10.4347	10.4423				
p-cymene	7463	99-87-6	12.7541	12.7591	12.7564	12.7623	12.7961	12.8524
1,8-cineole	2758	470-82-6	12.9866	12.9831	12.9765	12.9955	13.1048	13.1587
(1R)-camphor	159055	464-49-3	16.4987	16.4946	16.484	16.4817	16.4843	16.4933
(-)-β-pinene	440967	18172-67-3		11.3059	11.3053	11.3045	11.3042	11.3172
(+)-limonene	440917	5989-27-5		12.8847	12.8867	12.9022	12.961	
estragole	8815	140-67-0		18.002	18.0019	18.0018	17.9941	
tricyclene	79035	508-32-7			9.613	9.6247	9.6215	9.6338
(+)-α-pinene	82227	7785-70-8			9.9741	9.9848		
camphene	6616	79-92-5			10.4377	10.4389		
1-isopropyl-4-methylenebicyclo[3.1.0]hex-2-ene	524198	36262-09-6			10.6055	10.6082	10.607	10.6179
β-pinene	14896	127-91-3			11.3053			
myrcene	31253	123-35-3			11.6886	11.6919	11.6972	11.7096
3-carene	26049	13466-78-9			15.0224	15.0229	12.323	12.3344
borneol	64685	507-70-0			17.097			
α-terpineol	17100	98-55-5			17.7988	17.7971	17.7885	
cyclofenchene	79022	488-97-1				9.6251		
3-octanone	246728	106-68-3				11.561	11.5506	11.5588
α-thujene	17868	2867-05-2				12.1342	12.133	9.7954
α-terpinene	7462	99-86-5				12.5159	12.5191	
γ-terpinene	7461	99-85-4				13.8151	13.8104	13.8196
isoborneol	6321405	124-76-5				17.0978	17.0912	
terpinene-4-yl-acetate	20960	4821-04-9				17.4231		
(+)-borneol acetate	6950274	20347-65-3				20.5213		
3-hexen-2-one	5367744	763-93-9					6.2029	
α-phellandrene	7460	99-83-2					12.133	12.1442
2-methylbicyclo[4.3.0]non-1(6)-ene	578219	60223-07-6					10.4526	
(1S,3R,6R)-(-)-4-carene	530422	29050-33-7					14.7269	14.7326
2-m-tolylpropene	70759	1124-20-5					14.737	14.7463
(+)-3-carene	443156	498-15-7					15.0162	15.0223
pinocamphone	6427105	547-60-4					16.9527	
pinocarvone	121719	30460-92-5					17.0157	
piperityl acetate	102578	1204-30-4					17.4161	
(-)-bornyl acetate	93009	5655-61-8					20.5135	
β-caryophyllene	564746	242794-76-9					24.187	
toluene	1140	108-88-3						5.48

Table 9. *Cont.*

Compound Name (PubChem)	PubChem CID	CAS	Retention Time (Rt) of Identified Compounds at Different Agitator Temperatures (°C)					
			40 °C	50 °C	60 °C	70 °C	80 °C	90 °C
3-methylpent-3-en-2-one	79048	565-62-8						6.2061
bornylene	10047	464-17-5						9.1041
acetone	180	67-64-1						10.4325
terpinolene	11463	586-62-9						12.5338
o-cymene	10703	527-84-4						12.8531

Note: All compounds were identified according to their retention time and mass spectrometry data.

3.1. Basil Test Results

Based on the chromatograms obtained at 40 °C, it can be concluded that only 1,8-cineole and estragole (Rt = 12.9959 and 17.9963) components could be identified at the applied temperature at basil. The peak at the forepart of the chromatogram refers to no sample component, but an analytical background signal, which might be ascribed to the impurity leaving the column at the beginning of the analysis (Figure S1A; see Supplementary S1). As the agitator temperature increased, additional terpenes (e.g., α-pinene, camphene, and thymol) and oxygenated compounds appeared in chromatograms, so the number of identified compounds increased.

The retention time, PubChem ID, and CAS number of all identified compounds at different agitator temperatures in the basil sample are presented in Table 4. Increasing agitator temperature resulted in the detection of a broader range of volatile compounds. At 40–50 °C, only a few major constituents, such as estragole, 1,8-cineole, and p-cymene, were observed, whereas higher temperatures (60–90 °C) enabled the identification of additional monoterpenes, sesquiterpenes, and oxygenated derivatives. Retention times remained quite consistent across the tested range, but compound diversity and complexity increased notably at elevated temperatures.

The results show that at 50 °C (Figure S1B; see Supplementary S1), in addition to 1,8-cineole and estragole, p-cymene, 3-carene and -methyl cinnamate in the basil sample were already identified by the applied method. At a higher pretreatment temperature (70 °C) (Figure S1D), in addition to the components detected at lower temperatures, several other volatile compounds, like (1R)-camphor, thymol, limonene, β-myrcene, and some further derivatives could be identified. A total of 21 different compounds were identified at this temperature (Table 4).

At 80 °C, the decomposition of further volatile organic compounds and the formation of derivatives might be observed. A total of 35 different compounds were identified at this temperature (Table 4). At this pretreatment temperature, some new compounds appeared in the chromatogram (e.g., fenchone, γ-terpinene, humulene, and caryophyllene) and were also identified.

Compared to the previously presented results, at 90 °C, additional compounds like hexanal, hexenal, and copaene were determined by the simple headspace technique in the case of the basil sample (Figure S1F; see Supplementary S1).

From these results, it was accordingly found that the extraction efficiency increased with rising temperature, which facilitated the volatilization of compounds from the sample to the overlying HS. Similar results were obtained during optimization of the static headspace GC-MS method for the leaf volatiles of 42 citrus cultivars [37]. These findings suggested that the extraction efficiency of the VOCs at higher temperatures was higher

than at lower temperatures, indicating that temperature has an important influence on the extraction of VOCs.

3.2. Yarrow Test Results

The HS-GC-MS test results of the yarrow plant using different sample-preparation temperatures are demonstrated in the chromatograms (Figure S2; see Supplementary S2).

Similarly to basil, not many diverse compounds were detected at the lowest pretreatment temperature (40 °C). Only the estragole peak was identified (Rt = 18.0039) (Figure S2A; see Supplementary S2). This indicates that the sample-preparation temperature used was too low for the volatile organic compounds to be released in the vapor space above the sample.

At higher sample-preparation temperatures (50 °C and 60 °C), ten components were identified, e.g., 1,8-cineole, anethole, β-pinene, and p-cymene, which are typical volatile compounds for yarrow. Furthermore, a heptane and a hexene derivative could also be identified by the HS-GC-MS technique (Figure S2B,C; see Supplementary S2).

The retention time, PubChem ID, and CAS number of all identified compounds at different agitator temperatures in the yarrow sample are presented in Table 5.

At 70 °C, 26 distinctive volatile organic compounds could be identified (Figure S2D; see Supplementary S2 and Table 5). The most important of these substances are myrcene, cymene, limonene, 1,8-cineole estragole, and β-phellandrene. This indicates the starting decomposition of some of the major components of yarrow, as well as the effective release of volatile terpene derivatives at 70 °C.

The obtained chromatograms show that at 80 °C, even more compounds (39 different derivatives) are released (Table 5). Beyond the compounds detected at the previous temperatures, various hexa- and hepta-dienes, γ-terpinene, 3-carene, isoborneol, and terpinol were identified (Figure S2E; see Supplementary S2).

At an even higher temperature (90 °C), 35 different compounds could be identified by means of thermal sample pretreatment (Table 5). This means that at this temperature, the presence of additional compounds could be confirmed in the yarrow sample, such as hexanol, methyleugenol, and borneol (Figure S2F; see Supplementary S2).

The chromatograms illustrate that the vertical axis values increase with increasing pretreatment temperatures. This confirms the fact that increasing temperature has a positive effect on the release of components into the vapor space. This result is in agreement with related studies, which show that heat treatment of the samples can accelerate the molecular motion—hence the release of relevant analytical components from the sample—increase the vapor pressure, and improve the sensitivity [37,38].

Figure S3 represents the effect of increasing temperature on the components' peak area. From the total chromatogram, the chosen peak was 3-carene at 15.019 min retention time (see Supplementary S3). The increasing temperature results significantly enhanced peak area. Similar trends were also observed for other components (Table 6).

3.3. Thyme Test Results

The HS-GC-MS test results of the thyme plant using different sample treatment temperatures are presented in the chromatograms (Figures S4A–F; see Supplementary S4).

In the case of thyme, some VOCs (β-myrcene, α-pinene, γ-terpinene, p-cymene, 1,8-cineole, estragole, thymol, etc.) could be identified in the lower temperature range (40–60 °C) (Figure S4A–C; see Supplementary S4, Table 7).

When a higher temperature (70 °C) was applied, 21 distinctive derivatives were identified (Table 5). Beyond the compounds detected at the previous temperatures, various methyl esters, cyclohexene derivatives, isoborneol, and (1R)-camphor were identified by the applied HS method (Figure S4D; see Supplementary S4).

An increase in sample-preparation temperature of 10 °C allowed for the generation of additional volatile organic compounds. At this temperature (80 °C), aromatic, ester, phenol, and alcohol derivatives could be identified. In total, 29 different compounds were present in the reaction medium (Figure S4E; see Supplementary S4).

In addition to the compounds detected at the previous temperatures, some additional volatile compounds (mostly esters) were found in the chromatogram obtained when a pretreatment temperature of 90 °C was applied (in total, 37 different compounds were identified). This means that the application of higher temperatures contributes to increased vapor pressure and promotes release of analytes into the headspace [38,39].

3.4. Oregano Test Results

The HS-GC-MS test results for oregano plants using different sample-preparation temperatures are shown in the chromatograms (Figure S5A–F; see Supplementary S5).

At lower temperatures (from 40 °C to 60 °C), only some compounds (6–7 different VOCs), like α-pinene, 1,8-cineole, p-cymene, and limonene were identified in the oregano sample (Figure S5A–C; see Supplementary S5).

When we applied an increased temperature (70 °C) for sample pretreatment, 17 VOCs were identified (Figure S5D; see Supplementary S5, Table 8). Due to the elevated temperature, γ-terpinene, camphene, and (1R)-camphor could also be identified between 10 and 18 min of retention time.

The retention time and CAS number of all identified compounds at different agitator temperatures in the oregano sample are presented in Table 8.

The gained chromatograms obviously indicate that at 80 °C, 34 different derivatives were detected (Table 8). In addition to the compounds found at the previous temperatures, various heptane and cyclohexene derivatives, caryophyllene, and α-phellandrene were also identified (Figure S5E; see Supplementary S5).

The obtained results demonstrate that at 90 °C, the decomposition of distinctive volatile organic compounds and the formation of new derivatives might occur. A total of 41 different compounds were identified at this temperature in the sample (Table 8). Similarly to the previous temperatures, 1,8-cineole and estragole as main components were identified, as well as 4-carene, cyclohexane, two additional hexenal derivatives, and thujone, which were newly observed compounds at this temperature. At this temperature, the presence of α-terpineol was also detected (Figure S5F; see Supplementary S5, Table 8).

In sum, it can be concluded that in oregano (*Origanum vulgare* L.), low temperatures (40–50 °C) yielded only a few dominant volatiles, including α-pinene, D-limonene, estragole, 1,8-cineole, and p-cymene, while higher temperatures (60–90 °C) facilitated the detection of additional terpenes, sesquiterpenes (e.g., caryophyllene, β-bisabolene), and oxygenated derivatives such as α-terpineol and thymoquinone. Retention times remained highly consistent across all conditions, but compound richness and chemical diversity increased with rising agitator temperature.

3.5. Rosemary Test Results

The HS-GC-MS test results for rosemary plants using different sample-preparation temperatures are shown in the chromatograms (Figure S6A–F; see Supplementary S6). The retention time and CAS number of all identified compounds at different agitator temperatures in the rosemary sample are presented in Table 9.

Using the lowest agitator temperature, only 5 different volatile compounds, namely α-pinene, camphene, p-cymene, 1,8-cineole, and (1R)-camphor could be identified, while at 50 °C, some additional compounds like β-pinene, (1S)-, D-limonene, and estragole were also detected (Figure S6A–B; see Supplementary S6).

The chromatogram obtained at 60 °C indicates that the decomposition and transformation of the volatile organic compounds are initiated at this temperature. A total of 15 different components were identified at 60 °C (Table 10). This means that when applying 60 °C for the pretreatment, some new peaks appeared, such as camphene, α-terpineol, and myrcene (Figure S6C; see Supplementary S6).

At 70 °C, more additional volatile organic compounds appeared in the chromatogram (in total, 21 components were identified (Table 10)). Besides the compounds previously identified at 60 °C, isoborneol, α-pinene, and camphene were also detected (Figure S6D; see Supplementary S6).

Based on the chromatograms obtained at 80 °C, it can be concluded that at this sample-preparation temperature, the identification of additional compounds is possible. For example, a hexadiene derivative, pinocarvone, and α-phellandrene were also detected (Figure S6E; see Supplementary S6).

The chromatograms obtained when applying 90 °C for sample preparation allowed the identification of the previously described compounds in addition to o-cymene and toluene (Figure S6F; see Supplementary S6). This finding is in good correlation with earlier studies [37,39,40] stating that heating of the sample increased the efficiency of the identification.

3.6. Examination of Carry-Over Effect

The headspace (HS) gas chromatography technique is one of the most widely used methods for the determination of volatile organic compounds (VOCs) from various matrices, including plant samples [29,36]. Despite its high sensitivity and solvent-free nature, several potential sources of cross-contamination may arise during the HS-based analyses, which can significantly affect both the accuracy and the reproducibility of the measurements.

One of the most common issues is the "carry-over" effect, particularly in the case of concentrated samples. Certain VOCs may adhere to the internal surfaces of the syringe, valves, or injector, and subsequently be introduced into the sample, thereby reducing the measurement reproducibility. To clarify this effect, blank samples were analyzed after the 90 °C thermal pretreatment step. The chromatograms of these measurements are demonstrated in Figure S7 (see Supplementary S7).

From the chromatograms, it might be concluded that no cross-contamination was observed in any of the blank samples. This indicates that the final applied temperature ramp was effective in removing any residual deposits or contaminants from the system.

The peak at the forepart of the chromatograms refers to no sample component, as it was discussed earlier, but an analytical background signal, which derives from the column at the beginning of the analysis.

4. Discussion

Regarding current challenges related to GC-MS measurement of volatile compounds, in our study, we focused on the elaboration of a simplified sample-preparation procedure minimizing the time required for sample preparation and eliminating the use of organic solvents being hazardous to humans.

The main advantage of this method is that it involves a very limited sample-pretreatment step without the use of many solvents or expensive sample preparation. Plant material (fresh or dried) can be placed in the sample vial and analyzed directly. The only disadvantage of HS is the limitation of the analysis of volatile compounds [36].

Furthermore, the volatile compounds studied are highly sensitive to temperature, as they have a low decomposition temperature and mostly moderate thermal stability (Table 3).

However, due to the various sample preheating methods (adjustable agitator temp. and variability of temp. ramps), a large number of biologically active phytochemical compounds can be detected via the HS method without compromising the sensitivity of the components to be tested. Throughout our efforts in method development, the main aim was to investigate how thermal pretreatment patterns affect the detectability of the inherent components, as very few publications are available on this matter.

In the HS-GC-MS technique, thermal treatment of the sample is a critical step during the sample-preparation process. This step allows the volatile components of the sample to be released and transferred into the gas phase without triggering unwanted reactions or degradation. Therefore, it is particularly important to select and apply temperatures that facilitate the transfer of volatile components into the gas phase while avoiding thermal degradation. Therefore, the thermal treatment might be regarded as a particularly critical phase of the analysis, as it ensures the detectability of the appropriate volatile compounds, while minimizing the interference caused by non-volatile or degraded substances during the analysis.

The improved analytical approach in our study signified the untargeted volatilomics, as nowadays it is considered the most common technique used to profile plant volatilomes. The untargeted method emphasizes the detection of all detectable metabolites in the sample [41]. However, the method suffers from spectral convolution, low sensitivity, limited annotation coverage, and poor reproducibility [41–45].

In this study, we did not aim to quantitatively analyze the individual components. The main focus was placed on investigation of the effect of increasing pretreatment temperature on the number of detectable components. The numbers of volatile organic compounds identified at different sample-preparation temperatures for each plant species are shown in Table 10.

Similarly to earlier findings, our results show that for each of the tested plant species, the increase in the sample-preparation temperature leads to an enhanced number of the detected compounds [33,34]. While at 40 °C only some of the volatile compounds deriving from the plant samples were detected by the HS-GC-MS technique, at a higher temperature (50 °C), we were able to identify 6–12, at 60 °C, 7–15, at 70 °C, 15–26, at 80 °C, 27–39, and at 90 °C, 21–41 different compounds for each plant species, respectively (Table 10). The increasing number of peaks appearing in the chromatograms associated with increasing temperatures suggests that the elevated temperature plays a crucial role in the qualitative identification of volatile compounds, as has also been pointed out by other authors [35–37,41].

Table 10. Number of compounds identified for each plant sample as a function of preparation temperature (°C).

	Number of VOCs Identified at Different Pretreatment Temperatures (°C)					
Plant	**40**	**50**	**60**	**70**	**80**	**90**
Basil	2	5	7	**21**	35	39
Yarrow	1	10	8	**26**	39	35
Thyme	8	12	14	21	**29**	37
Oregano	7	6	7	**15**	34	41
Rosemary	5	8	**15**	21	27	21

In Table 10, the temperature values and the corresponding numbers of the detectable volatile compounds are presented. Numbers in bold refer to compounds deriving from the advanced decomposition processes. Accurate knowledge of the decomposition tem-

peratures is essential not only for the selection of the most appropriate analytical method, but also with respect to modeling real, practical conditions of the processing. As these plant samples are mostly used in high-temperature cooking processes, it is important to be aware of the potential release of new volatile compounds during processing by cooking or baking [46,47].

It can be clearly established that as the temperature increased, more compounds could be identified for each plant species tested. However, it can also be observed that equally intense degradation processes did not occur at the same temperature for each plant. For example, in the case of thyme, the release of new volatile organic compounds steaming from decomposition started at 40 °C, while for the other plants it became intensified above 70 °C [48,49].

It can also be concluded that the used direct measurement method for each plant species (sample preparation with rising heat profile and headspace technique) is suitable for the identification of the generated volatile organic compounds and their derivatives. Furthermore, it is clear from our results that the number of the identifiable compounds exhibits variation in terms of the type of the diverse plant species. Although in many cases the same compounds appear in the vapor space above the distinct plant species, it might be claimed that the compounds are characteristically typical of each plant species and can be grouped and clearly identified. Table 11 presents the most important identified compounds in the cases of the studied plant species.

The obtained results show that among the monoterpenes limonene, while among the sesquiterpenes caryophyllene and humulene, were identified by the direct HS-GC-MS method. Among the phenols, we identified thymol, eugenol, and carvacrol. In addition to these compounds, several alkenes and aromatic and unsaturated compounds (cymene, terpinene, camphene, limonene, isoborneol, etc.), as well as ketone, ester, and aldehyde derivatives were also detected [32–34,50,51].

Table 11. The main identified components in the plant samples tested.

Plant	Main Compounds
Basil	limonene, borneol, and benzene derivatives (e.g., estragole and eugenol)
Yarrow	1,8-cineole, limonene, estragole, cymene, myrcene, β-phellandrene
Thyme	cymene, terpinene, estragole, camphene, limonene, isoborneol, thymol
Oregano	1,8-cineole, cymene, terpinene, estragole, camphene, limonene
Rosemary	myrcene, 3-carene, terpineol, estragole, camphene, limonene, isoborneol, α-phellandrene

5. Conclusions

An improved HS-GC-MS method was developed for direct profiling of volatile organic compounds (VOCs) in solid medicinal herbs. The optimized procedure reduces sample-preparation time, eliminates organic solvents, and minimizes environmental impact, consistent with green analytical chemistry principles.

Thermal pretreatment at 70–90 °C enhanced the detection of key volatiles such as mono- and sesquiterpenes, phenols, and aldehydes, without compromising reproducibility. The method is adaptable and resource-efficient, supporting comprehensive metabolite profiling and integration with complementary analyses.

This approach enables the sustainable utilization of VOC-rich herbs and improved recovery of bioactive compounds for functional food and preservative applications. Importantly, the protocol supports several United Nations Sustainable Development Goals, including good health and well-being (SDG 3), industry innovation and infrastructure (SDG 9), and responsible consumption and production (SDG 12).

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app16021031/s1, Figure S1: A-F. HS-GC-MS chromatogram of basil sample applied 40 °C (A) −50 °C (B) −60 °C (C) −70 °C (D) −80 °C (E) and 90 °C (F) °C thermal pretreatment; Figure S2: A-F. HS-GC-MS chromatogram of yarrow sample applied 40 °C (A) −50 °C (B) −60 °C (C) −70 °C (D) −80 °C (E) and 90 °C (F) °C thermal pretreatment; Figure S3: The effect of temperature on the component area at 3-carene in yarrow sample; Figure S4: A-F. HS-GC-MS chromatogram of thyme sample applied 40 °C (A) −50 °C (B) −60 °C (C) −70 °C (D) −80 °C (E) and 90 °C (F) °C thermal pretreatment; Figure S5: A-F. HS-GC-MS chromatogram of oregano sample applied 40 °C (A) −50 °C (B) −60 °C (C) −70 °C (D) −80 °C (E) and 90 °C (F) °C thermal pretreatment; Figure S6: A-F. HS-GC-MS chromatogram of rosemary sample applied 40 °C (A) −50 °C (B) −60 °C (C) −70 °C (D) −80 °C (E) and 90 °C (F) °C thermal pretreatment; Figure S7: A-E. Blank HS-GC-MS chromatograms obtained after the analysis of investigated samples basil (A)—yarrow (B)—thyme (C)—oregano (D) and rosemary (E) at 90 °C thermal pretreatment.

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