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INTENSIFIED MICROWAVE-ASSISTED HYDRODISTILLATION PRODUCES *TRANS*-ANETHOLE-RICH FENNEL (*FOENICULUM VULGARE* MILL.) FRUIT ESSENTIAL OIL

Article Highlights

- The MWHD isolation technique used was less time-consuming and resulted in a higher oil yield
- *Trans*-anethole-rich and estragole-poor oil expressed better antimicrobial activity
- Produced *Foeniculi aetheroleum* is promising for different industrial applications

Abstract

Fennel fruit (Foeniculim vulgare Mill.) essential oil (FFEO) was isolated from disintegrated plant material by intensified microwave-assisted hydrodistillation approach (MWHD). Isolated FFEO, rich in trans-anethole and low in estragol, was obtained with a 30% higher yield after only 10 min compared to the FFEOs isolated via traditional hydrodistillation (HD). Besides significant differences in the chemical composition, the MWHD-obtained FFEO demonstrated superior antibacterial activity, while its anticandidal activity remained unchanged and identical to HD-obtained FFEOs. MWHD technique also required almost 18 times less electricity consumption and emitted less CO_2 *than the conventional HD, suggesting this approach is more viable for FFEO production on an industrial level. According to these results, the MWHD-obtained FFEO has the potential to be used in the food industry and packaging, for the production of nutraceuticals, biopharmaceuticals, bioherbicides, and cosmetics.*

Keywords: antimicrobial activity; essential oil; fennel fruit; Foeniculi aetheroleum; grinding; microwave-assisted hydrodistillation.

Due to increased consumers’ concerns regarding the safety of synthetic chemicals present in various foods and pharmaceutical products, a major shift in industrial research is focused on the replacement of these chemicals with “greener” and natural alternatives. Nowadays, one of these alternatives are essential oils (EOs) derived from different aromatic plants due to their various beneficial biological activities [1]. One example of a commercially valuable EO-bearing industrial crop is fennel (*Foeniculum vulgare* Mill., family Apiaceae). Although native to the Mediterranean and southern Europe, it is cultivated worldwide as a crop with great industrial and economic value, especially in the food and pharmaceutical industries [1]. Fennel is a traditionally highly-valued spice and medicinal herb; some of its reported biological activities include antibacterial, antifungal, anti-inflammatory, analgesic, anti-parasitic, antioxidant, antithrombotic, antidiabetic, antiulcer, antiseptic, antispasmodic, carminative, hepatoprotective and mosquito-repellent [2–6]. Mature fennel fruits (colloquially called seeds) are especially attractive and are extensively investigated as a valuable material in food products, cosmetic, pharmaceutical formulations, culinary additives, flavoring agents, insect- and pest-repellent products, perfumes, beverages, and confectionery [1,4,7]. Their unique aroma, characteristic flavor, and numerous biological activities are attributed to the isolated EOs,

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which have been shown to have antimicrobial, antioxidant, anti-inflammatory, antispasmodic, cytotoxic, and hepatoprotective activities [1,3,4,8–10]. Consequently, fennel fruit essential oil (FFEO), *i.e.* *Foeniculi aetheroleum* is widely used in many industrial areas (food, cosmetic, and pharmaceutical engineering) and is a promising safe and environmentally friendly bioherbicidal agent [11]; therefore demanding a need to design improved method for its extraction, utilization and global commercialization. FFEOs major compounds include *trans*-anethole, estragole, fenchone, limonene, α-pinene and α-phellandrene [9,12]. Remarkable differences in both the yield and constituents of FFEOs are ascribed to the factors related to the type of cultivated fennel plant (origin, genotype, development stage), cultivation conditions (location, climate, environmental conditions, agricultural practices), plant material pretreatment (powder size, soaking in solvent) and the extraction technique applied [4,7,13].

A crucial step in EO isolation is the selection of an adequate extraction technique that maximizes EO yield while minimizing changes in its functional properties [4]. Traditional, conventional, and most commonly employed EO isolation methods are hydrodistillation (HD), steam distillation, maceration, and expression [4]. However, these methods have some disadvantages: higher energy and solvent consumption, longer duration, lower EO extraction efficiency, yield, and even quality [14,15]. Moreover, some volatile constituents may be degraded due to prolonged extraction times, resulting in their loss and reducing the value of the obtained EO [5,14,15]. Employed toxic solvents that contaminate both the EO and the residual material are environmentally problematic [5,14]; their removal makes the technological process more expensive. Therefore, the focus of researchers is shifted towards the development of new and improved “greener” techniques to enhance the EO extraction efficiency, enable the acquirement of higher-quality EO, and overcome the listed disadvantages.

Microwave-assisted hydrodistillation (MWHD) for EO isolation as a “greener” technique offers numerous advantages compared to classical HD. MWHD is faster, more efficient, cost-effective, environmentally friendly, and surpasses direct plant material/heat source contact, thus ensuring faster and more effective heating, providing higher-quality EO [4,5]. The use of MWHD prevents the thermal decomposition of thermally labile and easily hydrolyzable EO components while improving EO separation [1,3]. Both distilled and indigenous water originating from the plant material serve as heat-transfer fluids [16]. Thanks to the mechanism of MWHD, one of the principal limiting steps regarding sample treatment in classical methods has been overcome. In classical methods, the EO extraction implies transferring the compounds into boiling water and their azeotropic distillation, thus requesting prolonged heating and stirring in boiling water. On the other side, MWHD is based on *in situ* water heating, causing the rupture of oil-containing cellular glands and easier EO release. This microwave superheating phenomenon *via* plant cell distension enables quicker EO liberation, compared to conventional steam distillation [16].

Many factors affect MWHD, *i.e.* quality, moisture content and particle size of raw plant material, nature and amount of solvent, pH, extraction duration, temperature, and microwave power [4]. Among these factors, the importance of milling is frequently addressed, since uncrushed plant material is less prone to EO release in higher yield. The particle size of the EO-bearing plant material reduced *via* physical milling (disintegration) results in increased surface area and enhances HD efficiency [9]. Milling provides a dual benefit regarding MWHD: solvent diffusion into the cells is enhanced, while cells’ rupture under microwave irradiation maximizes the extracted EO yield and enables the release of desired targeted components [1].

Among the main FFEO constituents, the dangers of specific alkenylbenzenes must be addressed, as their presence in larger quantities is considered both undesirable and problematic. Daily ingestion of plant-based supplements and products containing alkenylbenzenes, proven genotoxic and carcinogenic in animal studies, must be taken as a serious concern for human health and is, therefore, a high priority for risk management [17]. For instance, phenylpropanoid estragole (synonyms: 1-allyl-4-methoxybenzene; 1-methoxy-4-(2-propenyl)benzene; chavicyl methyl ether; estragon; isoanethole; methyl chavicol, *p*-allylanisole) stirred some controversies due to its genotoxic and carcinogenic effects on animal models [18], specifically as a weak hepatocarcinogenicity inducer in rodents [16]. Excessive FFEO heating causes isomerization of *trans*-anethole into estragole; some MWHD-obtained FFEOs had 5.5% higher estragole content [19]. Moreover, hepatotoxicity of several monoterpenes including camphor and limonene (also detected in FFEOs) was reported [20]. In a toxicological sense, high(er) intake of products containing mentioned compounds must be restricted, while their frequent ingestion in smaller amounts is still under controversial debate [18].

Due to increased global awareness, some consumers especially refuse to buy and consume estragole-containing products. Therefore, it is necessary to design estragol-free FFEOs' isolation processes, while simultaneously ensuring maximization of their yield and minimization of both time and power consumption.

In this work, the following steps are covered: (a) proposition of the "greener" intensified MWHD technique for obtaining *trans*-anethole-rich and estragole-poor FFEO; (b) chemical characterization of the isolated FFEO; (c) comparison of energy consumption, economic and environmental impacts of the proposed MWHD and traditional HD technique and (d) evaluation of the MWHD-obtained FFEO's antimicrobial activity, with comparison to those reported for FFEOs isolated *via* traditional HD.

MATERIALS AND METHODS

Plant material and chemicals

Raw and non-processed mature fennel fruits were picked and collected from the mountain field in Stara Planina, Serbia (43°22′10″N 22°36′32″E, collection period July 2023) and kept in a paper bag before usage at laboratory temperature (20 °C). The plant material was ground in an electric grinder (laboratory coffee mill Gorenje, Slovenia) for 60 s and sieved on a sieve shaker (0.5 mm fraction). Figure S1 (Supplementary materials) represents the visual comparison between raw and powdered fennel fruits. The moisture content of the raw and powdered fennel fruits was determined by drying in an oven at 105 °C to constant mass. The pulverized material was poured into a sealed dark glass flask (previously twice disinfected with 70% ethanol) and refrigerated at 4 °C in the dark. The pulverized samples were stored for 24 h.

Ethanol (70%, Zorka Pharma, Šabac, Serbia) and commercial distilled water (DCP Hemigal, Leskovac, Serbia) were used. Dimethylsulfoxide, diethyl ether, and anhydrous Na_2SO_4 (both ≥99%) were purchased from Sigma-Aldrich (Munich, Germany), while a sterile saline solution was from Hemofarm A.D. (Vršac, Serbia).

MWHD procedure

In a round-bottom one-necked glass flask 50 g of pulverized fennel fruit was mixed with 500 cm^3 of distilled water, attached to a Clevenger-type apparatus with a condenser, and subjected to microwave irradiation in a "Discover" microwave reactor (CEM, Matthews, North Carolina, USA). The total duration of the process was 20 min at 100 °C (operating frequency 2.45 GHz, power 500 W). Temperature control was performed by using an infrared mass measurement system and maintained constant during the process. The obtained FFEO was dehydrated with anhydrous Na_2SO_4, collected in a dark bottle, sealed, and stored at 4 °C prior to analyses. The FFEO yield was expressed in g of oil per 100 g of the plant material (g FFEO/100 g p.m.). The determination was performed three times and the data were expressed as mean ± standard deviation.

Identification of components

The qualitative and quantitative analyses (GC-MS: gas chromatography-mass spectrometry) and GC-FID: gas chromatography-flame ionization detection) conditions were identical to those described in Ilić et al. [9]. The determination was triplicated and the data were expressed as mean ± standard deviation.

Evaluation of energy demands and environmental impacts

Energy consumption during the MWHD process was calculated by equation:

$$E = P \cdot t \tag{1}$$

where E, P, and t denote the energy consumption, the applied power, and MWHD duration, respectively. The estimated quantity of the emitted CO_2 during MWHD was provided by the equation:

$$E_{CO_2} = 800 \cdot E = 800 \cdot P \cdot t \tag{1}$$

where E_{CO2} is CO_2 mass.

Antimicrobial tests

Antibacterial activity was tested against Gram(+) (*Staphylococcus aureus* ATCC 25923, *Listeria monocytogenes* ATCC 19118, *Bacillus subtilis* ATTC 6633) and Gram(-) bacteria (*Proteus vulgaris* ATCC 8427, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATTC 2785, *Salmonella typhimurium* CICC 10420). Anticandidal activity was evaluated against *Candida albicans* (ATCC 2091). The details of both the disc diffusion method and conditions used for minimal inhibitory concentration (MIC) determination were identical and detailed in Ilić *et al.* [9]. Tests were triplicated and the results were given as mean ± standard deviation.

Statistical analysis

The SPSS 23.0 (IBM, USA) software performed the analysis of variance (ANOVA) combined with Tukey's multiple comparison test (the significance level $p < 0.05$).

RESULTS AND DISCUSSION

FFEO yield, qualitative and quantitative composition

The determined moisture content of the raw and powdered fennel fruits was 5.43 ± 0.01 % and 5.33 ± 0.01 %, respectively.

During the first 10 min of MWHD, the FFEO yield rose exponentially, achieving 5.2 ± 0.01 g/100 g of plant material after 10 min. Further prolongation of the process had no significant impact on the FFEO yield, with a maximum value of 5.21 g/100 g of plant material after 20 min; therefore, 10 min was taken as the optimal time. MWHD-obtained FFEO yield after 10 min was 30% and 44.4% higher than the FFEO yield obtained by Clevenger-type HD from disintegrated and non-disintegrated fennel fruits after 3 h in our previous study [9]. MWHD enhances FFEO yield dually. Microwaves decrease the degree of oxidation and hydrolyzation by speeding the process and requiring lower solvent quantity while in the plant material heat is transferred from the center to the outside, causing higher pressure into the FFEO-bearing cells, resulting in their quick rupture [13].

By applying different modified MWHD methods, it is clear that a combination of different factors (the nature and origin of the fennel fruit, milling, moisture content, microwave power, solid-to-liquid ratio, and duration of the process) are crucial factors that determine the improvement in terms of the FFEOs yield, as well as their chemical compositions [1,3–5,13–16,21]. After pulverization, the smaller particle size of crushed fennel seed provides a higher surface area, thus more FFEO-bearing secretory canals are exposed [1,3–5,13]. The optimal moisture content of the raw material is especially beneficial. Higher moisture content leads to an increased dielectric heating rate, so FFEO release into the solvent is easier. The residual water contained in the fennel seeds evaporates rapidly, ensuring FFEO is extracted more quickly [4,13,14,21]. Microwaves make polar compounds in fennel fruits (including water) prone to ion conduction and dipole rotation, resulting in heating that disintegrates the plant cell membranes and FFEO release [4,13,14,21]. Precautions regarding excessive microwave power must be taken seriously since thermal degradation of the fennel fruits decreases the MWHD efficiency, FFEO yield, and quality [13,14,16]. An adequate solid-to-liquid ratio ensures the highest FFEO yield due to the appropriate dielectric heating of the solvent. Higher liquid quantity prolongs MWHD and produces unnecessary waste, while insufficient liquid poses a risk in raw material combustion and reduces the MWHD efficiency [13–16]. Sufficient MWHD duration is inevitable for the completion of the process and optimal release of each FFEO component, while prolonged irradiation decreases the FFEO yield and MWHD efficiency [1,4,13–16,21].

GC-MS and GC-FID chromatograms of FFEO isolated by MWHD are given in Figures S2 and Figure S3, respectively. Although the qualitative composition of FFEOs was identical, significant differences regarding the quantitative composition are noticed (Table 1).

Table 1. Comparison of FFEOs chemical composition (area %) isolated by MWHD and HD.

No.	Retention time, min	Compound	Type of compound	MWHD	HD, disintegrated fennel fruit [9]	HD, non-disintegrated fennel fruit [9]
1	4.90	α-Pinene	Monoterpene, bicyclic	<0.05 cG	2.04 ± 0.13 aE	0.51 ± 0.05 bE
2	5.10	Camphene	Monoterpene, bicyclic	<0.05 bG	0.16 ± 0.04 aI	0.05 ± 0.04 bF
3	5.36	Sabinene	Monoterpene, bicyclic	<0.05 aG	0.09 ± 0.04 aI	<0.05 aF
4	5.49	β-Myrcene	Monoterpene, acyclic	<0.05 cG	1.03 ± 0.04 aF	0.41 ± 0.04 bE
5	5.74	α-Phellandrene	Monoterpene, monocyclic	<0.05 cG	0.36 ± 0.04 aH	0.15 ± 0.04 bF
6	5.99	*p*-Cymene	Monoterpene, aromatic	<0.05 aG	0.10 ± 0.04 aI	0.09 ± 0.05 aF
7	6.06	Limonene	Monoterpene, monocyclic	3.08 ± 0.07 aC	2.32 ± 0.07 bD	1.09 ± 0.04 cD
8	6.45	γ-Terpinene	Monoterpene, monocyclic	0.75 ± 0.11 aE	0.72 ± 0.06 aG	0.38 ± 0.03 bE
9	6.91	Fenchone	Oxygenated monoterpene, bicyclic ketone	4.71 ± 0.06 cB	23.14 ± 0.06 aB	22.59 ± 0.05 bB
10	7.72	Camphor	Oxygenated monoterpene, bicyclic ketone	<0.05 bG	0.50 ± 0.07 aH	0.51 ± 0.04 aE
11	8.36	Estragole	Phenylpropanoid	<0.05 bG	2.58 ± 0.05 aC	2.67 ± 0.1 aC
12	9.09	*cis*-Anethole	Phenylpropanoid	0.99 ± 0.05 aD	0.12 ± 0.05 bI	0.18 ± 0.06 bF
13	9.15	*p*-Anisaldehyde	Phenylpropanoid	0.39 ± 0.05 aF	0.14 ± 0.06 bI	0.36 ± 0.04 aE
14	9.58	*trans*-Anethole	Phenylpropanoid	90.06 ± 0.06 aA	64.87 ± 0.07 cA	69.89 ± 0.06 bA
			Monoterpenes (1-8)	3.81 ± 0.108 bC	6.82 ± 0.366 aC	2.68 ± 0.231 cC
			Oxygenated Monoterpenes (9, 10)	4.71 ± 0.06 cB	23.64 ± 0.113 aB	23.1 ± 0.09 bB
			Phenylpropanoids (11-14)	91.4 ± 0.13 aA	67.71 ± 0.21 cA	73.1 ± 0.26 bA
			Total	99.92 ± 0.02 a	98.17 ± 0.68 b	98.88 ± 0.58 a,b

Different letters indicate statistically different (p <0.05) values in the same row (lower case letters) and in the same column (upper case letters) by Tukey's multiple range test.

Almost the total amount of MWHD-obtained FFEO was constituted of 14 volatile components, with *trans*-anethole as the most abundant (90%). Five minor components (less than 10% of FFEO) were in this order: fenchone > limonene > *cis*-anethole > *γ*-terpinene > *p*-anisaldehyde, while eight compounds were detected in traces (*α*-pinene, camphene, sabinene, myrcene, *α*-phellandrene, *p*-cymene, camphor and estragole). MWHD-obtained FFEO contained more *trans*-anethole, *cis*-anethole, and limonene, while fenchone was present in a lower quantity. As in the case regarding the FFEO yield, the nature and origin of the fennel fruit play a key role in many total compounds constituting the FFEO (Table 2).

Table 2. Literature review of the yield, MWHD conditions, and chemical composition of the main FFEO compounds from different origins.

Origin	Pretreatment method	Isolation method*	Operating conditions	Yield, %	Time, min	Number of components	Abundant compounds (%)	Reference
Serbia	Milling	MWHD	500 W, 0.5 mm particle size, liquid/solid ratio 10 mL/g, 100 °C	5.2±0.1	10	14	*Trans*-anethole (90.06 ± 0.06), fenchone (4.71 ± 0.06), limonene (3.08 ± 0.07), *cis*-anethole (0.99 ± 0.05), *γ*-terpinene (0.75 ± 0.11), *p*-anisaldehyde (0.39 ± 0.05)	This paper
Algeria	Freeze-drying, 48 h or raw seeds, milling	MWHD	305.9282 W, raw non-dryed seeds, 333.4673 μm particle size, water pH 6, liquid/solid ratio 6.77 mL/g	0.8999±0.02	20	19	*Trans*-anethole (88.7 ± 0.02), camphor (4.1 ± 0.03), estragole (2.9 ± 0.03), anisketone (0.6 ± 0.02), limonene (0.2 ± 0.01), fenchone (0.2 ± 0.01)	[4]
Mediterranean Region	Milling	MWHD	800 W, 10 min + 500 W, 50 min	2.8	60	4	*Cis*-anethol (65), fenchone (28), limonene (2.2), pinene (1.5)	[5]
Turkey	Grinding	MWHD	10 min, 800 W, 100 °C + 50 min, 500 W, 100 °C	1.6	60	34	*Trans*-anethole (85.2), estragole (4.7), limonene (4.1), α-fenchone (1.5), anisaldehyde (1.5), carvone (1)	[16]
	None			1.2	60	34	*Trans*-anethole (85.7), estragole (4.9), limonene (2.2), α-fenchone (1.4), anisaldehyde (2.4), carvone (1.8)	
China	Drying at room temperature, milling, soaking in water	DCMAHD	576 W, soaking time 1.82 h, 60-80 mesh particle size, liquid/solid ratio 8.69 mL/g	2.82±0.09	34	27	*Trans*-anethole (88.28), estragole (4.25), *D*-limonene (2.04), fenchone (2.03)	[1]
India	Drying	Coaxial MWHD	500 W, 9 min, 250 rpm + 300 W, 120 min, liquid/solid ratio 5 mL/g	0.83	120	17	*Trans*-anethole (79), fenchone (10.9), estragole (4.2), limonene (3.2)	[21]
Tunisia	Drying, milling, soaking in water at room temperature	ESFME	600 W, soaking time 20 min, particle size <2 mm, liquid/solid ratio 5 mL/g	0.48	30	9	*Cis*-anethole (83.07), *α*-terpinolene (10.9) eucalyptol (2.95), *γ*-terpinene (2.11)	[3]
		SFME		0.46	37	7	*Cis*-anethole (83.63), *α*-terpinolene (11.42), γ-terpinene (2.17), eucalyptol (2.12)	
China	Soaking in 73.2% [C$_4$MIM]Br, 240 W, 6 min	MILT-HD	240 W, liquid/solid ratio 6 mL/g, 100 ℃	0.0363 mL/g	76.2	11	Anethole (48.29), estragole (28.71), *D*-Limonene (8.32), fenchone (1.82), γ-terpinene (1.53), α-pinene (1.25), anisic aldehyde (0.83)	[15]
North Gujarat	Milling, soaking in water	IMAE	140 W, 0.4 mm particle size, 500 g material + 20 g carbonyl Fe powder (99.16% Fe content, 3.5 μm)	0.5	25	9	*Cis*-anethole (70), *L*-fenchone (6), α-limonene (3.1), myrcene (1.3), methyl chevicol (1.2), γ-terpenene (1.1), anisic aldehyde (1), caprinone (0.9)	[14]
China	Milling, drying at 40 ℃ for 48 h	DCME	600 W, 40-75 mesh particle size, liquid/solid ratio 5.6 mL/g	1.172±0.011	27	25	Estragole (64.25), limonene (10.11), fenchone (5.15), *trans*-anethole (3.98), *D*-carvone (1.75), fenchyl acetate (1.01)	[13]
	Milling, soaking in water for 8 min	SCME	600 W, liquid/solid ratio 5.6 mL/g	1.068±0.011	27	26	Estragole (58.61), limonene (13.06), fenchone (5.08), *trans*-anethole (4.84), *D*-carvone (1.77), fenchyl acetate (1.27)	

*MWHD, microwave-assisted hydrodistillation; DCMAHD, double-condensed microwave-assisted hydrodistillation; Coaxial MWHD, coaxial microwave-assisted hydrodistillation. * ESFME, enhanced solvent-free microwave extraction using double walled microwave reactor; SFME, solvent-free microwave extraction using the single-walled reactor; MILT-HD, microwave-assisted ionic liquids treatment followed by hydrodistillation; IMAE, improved microwave-assisted extraction; DCME, dual-cooled microwave extraction; SCME, single-cooled microwave extraction.

The applied MWHD approach was selective for producing phenylpropanoid-rich FFEO, followed by oxygenated monoterpenes and lastly monoterpenes. This is explained by differences in their dipolar moments. Phenylpropanoids and oxygen-containing constituents have higher dipolar moments than monoterpene hydrocarbons, causing more vigorous interaction with microwaves and facilitating their isolation *via* microwave extraction [3,4]. In that sense, *trans*-anethole, as an oxygenated compound with a higher dipolar moment, was extracted more easily, as a consequence of the MWHD-specific heating mechanism. Since the microwaves' electromagnetic energy is converted into heat through two mechanisms (ionic conduction and dipole rotation), alignment of the molecules with a dipole moment in the electric field causes frequent changes of direction, leading to particles' collisions and resulting in the liberation of heat energy. This ultimately causes the enhanced breakdown of hydrogen bonds by the dipole rotation of the molecule, enhancing the extraction process [22]. Regarding MWHD, the application of water as a microwave irradiation absorber is crucial, since inadequate or insufficient water content cannot reduce or prevent undesirable and harmful chemical reactions (such as hydrolyzation, oxidation, and degradation of volatile compounds), which do not occur during conventional hydrodistillation [3].

From the industrial point of view, phenylpropanoids and oxygen-containing constituents are more valuable and desirable since these compounds are more fragrant and beneficial for the production of EOs that are more olfactively appealing to consumers [3,4,13,16]. MWHD was found especially beneficial for extracting larger quantities of flavourful and fragrant fenchone, whose presence in EOs, and nutritional, cosmetic, and pharmaceutical products increases their market value [21]. The amount of fenchone, bicyclic ketone, was shown to increase with the MWHD duration time, therefore its amount in FFEO produced after 120 min is much higher [21] than in our study. Therefore, strict control regarding MWHD time consumption is crucial for obtaining FFEO rich in specific compounds (phenylpropanoids or oxygenated monoterpenes). According to Koşar et al. [16] crushed fennel fruits were found to be limonene-richer than raw fruits; however, contrary to this study, the applied "shock-and-treat" MWHD technique enabled adequate microwave energy intensity for limonene extraction from the plant matrix.

Some Mediterranean [5], Indian [14], Tunisian [3], and Chinese [15] MWHD-obtained FFEOs had fewer compounds than the isolated Serbian FFEO, while some FFEOs from India [21], Algeria [4], China [1,13] and Turkey [16] were composed of more substances. *Trans*-anethole was also the most prevalent constituent, as well as containing fewer amounts of estragole, in MWHD-obtained FFEOs from Algeria [4], China [1], Turkey [16], and India [21]. *Cis*-anethole was the most prevalent in Tunisian [3], Indian [14], and Mediterranean [5] MWHD-obtained FFEOs, while the Chinese FFEOs contained high amounts of estragole [13,15].

Estimation of the energy and environmental impact of the proposed MWHD technique

To estimate the possibility of applying the proposed MWHD technique on an industrial level, factors regarding energy demands and environmental impact are presented in Table 3.

Table 3. Comparison of the proposed methodology.

Factor	Unit	MWHD	HD, disintegrated fennel fruit	HD, non-disintegrated fennel fruit
Electric power	W	500	500	500
Temperature	K	373.15	373.15	373.15
Thermal conductance	kW/K	0.00134	0.00134	0.00134
Duration	min	10	180	180
Liquid-to-solid ratio	mL/g	10	10	10
Electricity consumption	kW·h	0.0835	1.5	1.5
E_{CO2}	g CO_2	66.8	1200	1200
Heat capacity	kW·h/K	0.000224	0.00402	0.00402
Mass of water per g p.m.	kg	0.01	0.01	0.01
Specific heat capacity	kW·h/kg·K	0.0224	0.402	0.402
FFEO yield	g/100 g d.w.	5.2 ± 0.1 a	4 ± 0.2 b	3.6 ± 0.3 b
FFEO yield per kW·h	g/100 g d.w./kW·h	62.275 ± 1.2 a	2.67 ± 0.13 b	2.4 ± 0.2 b
FFEO yield per kW·h·K	g/100 g d.w./kW·h·K	0.167 ± 0.0032 a	0.00715 ± 0.00036 b	0.0064 ± 0.00054 b

Row values (different small letters) are significantly different ($p < 0.05$) by Tukey's multiple range tests.

Considering that microwave heating is uniform and quicker compared to classical heating, combined with the fact that the cellular heating is caused by the moisture present in the plant tissue (thus produced vapor pressure disrupts the cellular walls), it can be concluded that the extraction of the components is both facilitated and accelerated. Reduction in solvent consumption and extraction time, together with lower energy consumption, are undoubtedly environmental advantages, establishing the MWHD as a "green" extraction technique [22,23]. Besides the fact that water is one of the most suitable solvents for microwave extraction, the potential of parameters' (time, power, and temperature) control, the possibilities of stirring and high-temperature application, and excluding the need for adding drying agents (since water absorbs microwaves) are also additional advantages worth mentioning [22,23]. So, the reduced cost of the proposed MWHD technique is beneficial in terms of time, energetic demands, and environmental impacts. MWHD technique provided a higher FFEO yield after only 10 min, compared to the 3 h required for conventional HD. MWHD technique also required almost 18 times less electricity consumption than the conventional HD; thus emitted less CO_2. For 1 kW·h 23.3 times more FFEO yield was produced via the MWHD technique, concluding this method is fast, cleaner, and promising for the production of *trans*-anethole-rich and estragole-poor FFEO on a larger industrial scale. The isolated FFEO has great potential regarding its utilization as a natural antimicrobial agent. It can be used to prolong the shelf life of meat since commercial fennel EOs have already been proven as natural antimicrobial agents [24]. The combination of EOs isolated from plants of different origins and the synergistic activity of their main and minor compounds is very interesting, opening a wide research field focused on utilizing FFEO as a potential natural preservative for different types of food. FFEOs' broader spectrum of potential application has been addressed, more specifically as an anticorrosive agent in the metallurgy, machine, automobile, and similar industrial branches, since these oils suppress corrosion of the steel plates [25].

Besides valuable FFEO, the suggested process also generates condensed residual herbal water and solid plant residual material, both of which can be further valorized into market-desirable products. Since residual water contains non-volatile organic bio-components, it can be considered aromatic water, thus applicable in various industries [26]; left-over plant material, constituted of insoluble cellulose-based components, represents biomass feedstock for the production of different chemicals, energy, animal food, and biofertilizers [27]. In this manner, the whole technological process can be scaled up on an industrial level, making it more profitable.

Antimicrobial activities

C. albicans was the most sensitive microorganism because it was completely cleared from a petri dish after treatment with the MWHD-obtained FFEO. Identical results were reported for previously HD-obtained FFEOs [9]. However, the MWHD-obtained FFEO showed better antibacterial activity compared to HD-obtained FFEOs (Table 4). Among Gram(+) bacteria, *B. subtilis* was the most sensitive, while the inhibition zone values for *S. aureus* and *L. monocytogenes* were similar. Some Gram(-) bacteria were also sensitive, namely *S. typhimurium*, *E. coli* and *P. vulgaris*. The lowest sensitivities were reported for *P. aeruginosa* and *K. pneumoniae*.

Table 4. Zones of bacterial growth inhibition (mm) caused by FFEOs isolated by MWHD and HD.

Microorganism	MWHD	HD, disintegrated fennel fruit [9]	HD, non-disintegrated fennel fruit [9]
S. aureus	28 ± 0.8 a C,D	23 ± 0.5 b B,C	19 ± 0.8 c B
L. monocytogenes	29 ± 0.9 a C	24 ± 1.1 b B	18 ± 1.0 c B,C
B. subtilis	34 ± 0.9 a A	32 ± 1.1 a A	28 ± 0.9 b A
P. vulgaris	28 ± 1.3 a C,D	23 ± 0.8 b B,C	19 ± 1.3 c B
E. coli	30 ± 0.6 a B,C	20 ± 1.1 b D,E	18 ± 0.6 b B,C
K. pneumoniae	26 ± 0.5 a D,E	21 ± 1.4 b C,D,E	19 ± 0.5 b B
P. aeruginosa	25 ± 1.0 a E	19 ± 0.7 b E	16 ± 1.0 c C
S. typhimurium	32 ± 0.5 a A,B	22 ± 0.8 b B,C,D	16 ± 0.8 c C

Different letters indicate statistically different ($p < 0.05$) values in the same row (lower case letters) and in the same column (upper case letters) by Tukey's multiple range test.

The lowest MIC values for *C. albicans*, *B. subtilis*, and *S. typhimurium* reflect the highest susceptibility of these pathogens, followed by *S. aureus* and *P. vulgaris* (Table 5). When MWHD-obtained FFEO was used, all MIC values were lower than those reported for previously HD-obtained FFEOs [9]. According to these results, MWHD-obtained FFEO can be utilized as a safer additive in food products, beverages, pharmaceuticals, nutriceuticals, and cosmetic formulations, preventing microbial growth, spoilage, and oxidative deterioration.

FFEO was previously reported as antifungal against *C. albicans* [28], *C. gloeosporioides*, *P. capsici*, *S. sclerotiorum,* and *F. fujikuroi* [1] and antibacterial against *S. typhimurium*, *E. coli*, *L. monocytogenes* and *S. aureus* [13]. FFEOs' constituents that are both of lower lipophilicity and mass penetrate the fungal cell membrane more easily, resulting in its disintegration; this explains FFEOs' prominent and diverse fungicidal activities [1]. Contrary to Gram-positive bacteria, weaker FFEO's antibacterial activity against Gram-negative bacteria is ascribed to their protective lipopolysaccharide-containing external membrane [29]. MWHD-obtained FFEO's improved antimicrobial activity can be ascribed to its specific chemical composition. The most abundant *trans*-anethole and minor-present fenchone, limonene, estragole, and *α*-pinene in their pure form were established as excellent antimicrobials [12,29]. In addition, synergistic effects occurring among *trans*-anethole and minor components may contribute to FFEO's better antimicrobial activity.

Table 5. MIC values (μg/ml) for FFEOs isolated by MWHD and HD.

Microorganism	MWHD	HD, disintegrated fennel fruit [9]	HD, non-disintegrated fennel fruit [9]
S. aureus	20 aB	50 bC	75 cC
L. monocytogenes	50 aE	90 bE	Non-treated
B. subtilis	15 aA	25 bA	25 bA
P. vulgaris	30 aC	50 bC	50 bB
E. coli	40 aD	75 bD	100 cD
K. pneumoniae	50 aE	75 bD	100 cD
P. aeruginosa	75 aF	>100 bF	>100 bE
S. typhimurium	15 aA	30 bB	Non-treated
C. albicans	15 aA	25 bA	25 bA

Different letters indicate statistically different ($p < 0.05$) values in the same row (lower case letters) and in the same column (upper case letters) by Tukey's multiple range test.

CONCLUSION

MWHD technique at 500 W power for 10 min (intensified "shock-and-treat" approach) resulted in obtaining *trans*-anethole-rich and estragole-poor FFEO from disintegrated fennel fruits while demanding less energy. Considering grouped compounds, FFEO contained more than 90% phenylpropanoids, less than 5% oxygenated monoterpenes, and close to 4% monoterpene hydrocarbons. Besides retaining its anticandidal potency, MWHD-obtained FFEO showed better antibacterial activity against *B. subtilis*, *S. aureus*, *L. monocytogenes*, *S. typhimurium*, *E. coli*, *P. vulgaris*, *P. aeruginosa*, and *K. pneumoniae*, compared to HD-obtained FFEOs. These results suggest that the MWHD-obtained FFEO may be considered as safer antimicrobial and spoilage-preventive additive for different industrial products. Moreover, the proposed technique is "green", safe, cheap, simple, effective, quick, and selective, thus promising for EO isolation from other aromatic plants.

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LIST OF SYMBOLS AND ABBREVIATIONS

Coaxial MWHD	coaxial microwave-assisted hydrodistillation
DCMAHD	double-condensed microwave-assisted hydrodistillation
DCME	dual-cooled microwave extraction
EO	essential oil
ESFME	enhanced solvent-free microwave extraction using a double-walled microwave reactor
FFEO	fennel fruit essential oil
HD	hydrodistillation
IMAE	improved microwave-assisted extraction
MIC	minimal inhibitory concentration
MILT-HD	microwave-assisted ionic liquids treatment followed by hydrodistillation
MWHD	microwave-assisted hydrodistillation
SCME	single-cooled microwave extraction
SFME	solvent-free microwave extraction using the single-walled reactor

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INTENZIVNIJA MIKROTALASNA HIDRODESTILACIJA U PROIZVODNJI ETARSKOG ULJA PLODA KOMORAČA (*Foeniculum vulgare* Mill.) VISOKOG SADRŽAJA *TRANS*-ANETOLA

Etarsko ulje ploda komorača (Foeniculim vulgare Mill.) izolovano je iz samlevenog biljnog materijala postupkom intenzivne mikrotalasne hidrodestilacije. Ovim postupkom izolovano ulje, visokog sadžaja trans-anetola i manjeg sadržaja estragola, dobijeno je nakon samo 10 minuta i u prinosu većem za 30% u odnosu na prinos ulja dobijeno postupkom konvencionalne hidrodestilacije. Pored značajnih razlika u hemijskom sastavu, ulje dobijeno mikrotalasnom hidrodestilacijom pokazalo je snažniju antibakterijsku aktivnost od ulja dobijenog konvencionalnom hidrodestilacijom, dok je antikandidijalna aktivnost ostala nepromenjena. Postupkom intenzivne mikrotalasne hidrodestilacije zabeležena je oko 18 puta manja potrošnja električne energije, kao i manja emisija CO_2 *u poređenju sa postupkom konvencionalne hidrodestilacije, što ukazuje da je ovaj postupak prihvatljiviji za proizvodnju etarskog ulja na industrijskom nivou. Prema ovim rezultatima, etarsko ulje komorača izolovano postupkom intenzivne mikrotalasne hidrodestilacije se može koristiti u prehrambenoj industriji i pakovanju, za proizvodnju nutraceutika, biofarmaceutika, bioherbicida i kozmetičkih preparata.*

Ključne reči: antimikrobna aktivnost; etarsko ulje; plod komorača; Foeniculi aetheroleum, mlevenje; mikrotalasna hidrodestilacija.

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