applied sciences

MDPI

Article

Chemical Composition and Antimicrobial Activity of Lavender (*Lavandula angustifolia* Mill.), Peppermint (*Mentha piperita* L.), Raspberry Seed (*Rubus idaeus* L.), and Ylang-Ylang (*Cananga odorata* (Lam.) Essential Oils—Towards Hurdle Technologies in the Production of Chocolate Mousse

Zapryana Denkova [1], Bogdan Goranov [1], Denica Blazheva [1], Teodora Tomova [1], Desislava Teneva [2], Rositsa Denkova-Kostova [3,*], Aleksandar Slavchev [1], Rafael Pagán [4], Pascal Degraeve [5] and Georgi Kostov [6]

[1] Department "Microbiology", University of Food Technologies, 26 Maritza Boulevard, 4002 Plovdiv, Bulgaria; zdenkova@abv.bg (Z.D.); b_goranov@uft-plovdiv.bg (B.G.); d_blazheva@uft-plovdiv.bg (D.B.); teodoratomova1211@gmail.com (T.T.); a_slavchev@uft-plovdiv.bg (A.S.)

[2] Laboratory of Biologically Active Substances, Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, 139 Ruski Boulevard, 4000 Plovdiv, Bulgaria; desislava.teneva@orgchm.bas.bg

[3] Department "Biochemistry and Molecular Biology", University of Food Technologies, 26 Maritza Boulevard, 4002 Plovdiv, Bulgaria

[4] Departamento de Producción Animal y Ciencia de los Alimentos, Facultad de Veterinaria, Instituto Agroalimentario de Aragón-IA2, Universidad de Zaragoza-CITA, Calle Miguel Servet, 177, 50013 Zaragoza, Spain; pagan@unizar.es

[5] Bioingénierie et Dynamique Microbienne aux Interfaces Alimentaires Research Unit, IUT Lyon 1, Technopole Alimentec, Université Claude Bernard Lyon 1, ISARA Lyon, 155 rue Henri de Boissieu, 01000 Bourg en Bresse, France; pascal.degraeve@univ-lyon1.fr

[6] Department "Technology of Wine and Beer", University of Food Technologies, 26 Maritza Boulevard, 4002 Plovdiv, Bulgaria; george_kostov2@abv.bg

* Correspondence: rositsa_denkova@uft-plovdiv.bg; Tel.: +359-899-08-55-20

Abstract: The growing consumer demand for the development of functional foods with a number of benefits for the consumer has led to a considerable increase in the studies focused on examining different natural agents to be included in the composition of newly developed functional foods. The chemical compositions of the essential oils (EOs) of lavender (*Lavandula angustifolia* Mill.), peppermint (*Mentha piperita* L.), raspberry seed (*Rubus idaeus* L.), and ylang-ylang (*Cananga odorata* (Lam.)) were determined using gas chromatography with a mass selective detector (GC-MS). The antibacterial and antifungal activities of these EOs were examined using a high-throughput 96-well microplate bioassay procedure and the MICs of each EO against each test microorganism were determined. The results indicated significant antimicrobial activity against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 6538, *Salmonella abony* NTCC 6017, *Pseudomonas aeruginosa* NBIMCC 1390, *Bacillus subtilis* ATCC 19659, *Penicillium chrysogenum* ATCC 28089, *Fusarium moniliforme* ATCC 38932, *Aspergillus niger* ATCC 1015, and *Aspergillus flavus* ATCC 9643. To explore their potential applications in food preservation, model chocolate mousse food emulsions were prepared that incorporated EOs and/or selected probiotic lactobacilli strains in both free and encapsulated forms. The inclusion of EOs and/or probiotic lactobacilli resulted in enhanced microbial safety and an extended shelf life. Furthermore, the chocolate mousse variants that were biopreserved with the inclusion of probiotic lactobacilli maintained a high viable lactobacillus cell concentration throughout the storage period. As a result, these products would not only be suitable as functional probiotic foods but also as effective delivery vehicles for probiotic lactobacilli.

Keywords: essential oils; lavender; peppermint; raspberry; ylang-ylang; probiotic lactobacilli; biopreservation; chocolate mousse; functional food

Citation: Denkova, Z.; Goranov, B.; Blazheva, D.; Tomova, T.; Teneva, D.; Denkova-Kostova, R.; Slavchev, A.; Pagán, R.; Degraeve, P.; Kostov, G. Chemical Composition and Antimicrobial Activity of Lavender (*Lavandula angustifolia* Mill.), Peppermint (*Mentha piperita* L.), Raspberry Seed (*Rubus idaeus* L.), and Ylang-Ylang (*Cananga odorata* (Lam.) Essential Oils—Towards Hurdle Technologies in the Production of Chocolate Mousse. *Appl. Sci.* **2023**, *13*, 11281. https://doi.org/10.3390/app132011281

Academic Editor: Monica Gallo

Received: 16 September 2023
Revised: 9 October 2023
Accepted: 11 October 2023
Published: 13 October 2023

1. Introduction

According to the definition, "tailor-made foods" are foods that are produced for a special target group of consumers. These foods have different functional characteristics, that are given both by the raw materials that are used for food production and by the production methods [1,2]. To obtain this type of food, four main stages are followed; the identification of the chemical, physical, biological, nutritional, and other characteristics of the main ingredients [3] is the first one. Another important stage in the production of this food type is the application of so-called minimal-processing technologies to ensure their quality. This means applying minimal steps to preserve the product in order for the product to retain more of its functional properties [4–7]. One of these methods is the so-called hurdle technologies—the combination of separate types of hurdles to prevent microbial spoilage via the application of physical and biological factors [5]. The first step towards combining two types of biological hurdles, those of microbiological origin and biomolecules (EOs) with an emphasis on the chemical composition and biological value of the EOs, has been studied in the present manuscript.

Essential oils (EOs) are aromatic liquids derived from various plant materials, such as leaves, flowers, roots, and fruits. They serve as alternatives to commonly applied antibiotics and are used as food biopreservatives [8–14]. These oils are typically obtained via water distillation [15–17] and contain a complex mixture of volatile secondary metabolites with small molecular mass, which contribute to their distinct and specific aroma [18,19]. Humans have employed these plant compounds for thousands of years, not only as ingredients in perfumes or food additives but also as alternative medicines due to their biological properties, including antimicrobial and antioxidant activities [15–17,20,21]. These properties are closely related to their components, which mostly include terpene derivatives and oxygen-containing compounds [1,11,16–18]. Each component of an EO offers specific benefits to the body, with monoterpenoids exhibiting analgesic, insecticidal, diuretic, antiviral, antimicrobial, antioxidant, anti-inflammatory, immunomodulatory, and other actions [22–24]. EO composition is influenced by the plant variety and the pedoclimatic conditions in which it is grown [22].

Due to their antimicrobial properties, EOs are being used as alternatives to antibiotics and in combination with them to combat multidrug-resistant bacteria. They exhibit a broad spectrum of antimicrobial activity, affecting various microorganisms such as bacteria, viruses, yeasts, and fungi [25]. The increase in antibiotic resistance among microorganisms poses a significant threat to human health, partly due to the widespread and often improper use of antimicrobial chemicals and the excessive use of chemical preservatives in food, leading to undesirable consequences such as dysbiosis and anaphylactic shock [25]. Consequently, the search for new antimicrobial agents without adverse effects on the body has led to the application of EOs from plants with antimicrobial activity in recent years. Notable examples include lavender, peppermint, raspberry, ylang-ylang, and others [26,27] and their potential application in food biopreservation strategies. The inclusion of EOs in the composition of foods ensures the microbial safety of the resulting product but very high EO concentrations are actually detrimental to the product's sensory qualities. Thus, biopreservation strategies with the inclusion of EOs actually address the balance between food safety and preservation and improvement of the food sensorial characteristics. It has been established that EOs can be included in food formulations at concentrations up to 1% without negatively affecting the organoleptic characteristics of the resulting product [28].

Lavandula (common name lavender) is a genus of plants belonging to the Lamiaceae family. Among the various species, *Lavandula angustifolia* Mill. (formally *Lavandula officinalis*) is the most widely utilized. Lavender EO's color is yellow-green. This EO possesses a robust and distinct aroma, albeit slightly different from the fragrance of the flowers themselves. The composition of lavender EO includes notable compounds such as linalyl acetate, linalool, geraniol, borneol, cineole, pinene, camphor, and coumarin. Of particular value is the presence of linalyl acetate, which can range from 30% to 60% depending on the growing conditions. Lavender EO finds widespread usage in culinary, cosmetic, and medicinal applications because of its soothing, antiseptic, antidepressant, and anti-inflammatory properties [29,30].

Raspberry (*Rubus idaeus* L.) is a perennial bushy plant, both wild and cultivated, belonging to the Rosaceae family. Raspberry seed oil is rich in vitamin E, with significant amounts of α-tocopherol (12.6 mg/100 g) and γ-tocopherol (19.4 mg/100 g). It also contains carotenoids, which help prevent UV damage and promote cell regeneration, and essential fatty acids (linoleic acid (Ω-6) and α-linolenic acid (Ω-3)). Among the highly volatile components, the main compounds found in raspberry EO are α-pinene (25.3–27.0%), sabinene (17.6–19.6%), myrcene (12.3–14.4%), limonene, and terpinen-4-ol. This EO finds applications in both the food industry and medicine because of its potent anti-inflammatory properties [31–33].

Ylang-ylang (*Cananga odorata* (Lam.)) Hook.f. & Thomson belongs to the Anonaceae family. Ylang-ylang EO contains: geraniol, terpineol, nerol, limonene, ylangol, linaloacetate, linalool, eugenol, caryophyllene, geranyl acetate, germacrene, p-cresyl methyl ether, benzyl benzoate, methyl benzoate, benzyl acetate, sesquiterpenes, monoterpenes, phenylpropanoids. The main aroma components of ylang-ylang oil are linalool, benzyl acetate, methyl benzoate, and p-cresyl methyl ether [34]. Ylang-ylang is used in herbal medicine, cosmetics, and medicine to treat malaria, stomach diseases, asthma, gout, and rheumatism [34,35].

Mentha is a genus of perennial herbaceous plants that is part of the Lamiaceae family. It is primarily found in the northern temperate zone. *Mentha piperita* L. (peppermint) contains around 1–3% EO, which includes components such as menthone, terpenes, isovaleric acid esters, and menthol (comprising 45–65% of the oil composition). Other constituents found in peppermint EO include amyl alcohol, aldehydes, tannins, enzymes, resins, ursolic acid, carotenoids, hesperidin, mineral salts (such as calcium, magnesium, and iron), and carotene. Notably, perillyl alcohol, a phytonutrient and monoterpene, is abundant in this herb oil. High-quality peppermint oils typically contain 50–60% menthol, with approximately 20% in ester form. The key constituents necessary for oil quality include the ketones jasmone and menthone, limonene, menthofuran, neomenthol, isomenthol, pulegone, pinene, piperitone, and cineole [36–38]. Peppermint also contains other beneficial substances such as L-carvone, flavone glycosides, vitamin C, vitamin B5, manganese, folate, and dietary fiber. The leaf mass of peppermint contains tannins (approximately 6–12%), bitter substances, flavonoids, nicotinic acid, and nicotinic acid amide. Peppermint finds applications in the food industry, as well as in pharmacy and medicine. It is an ingredient in various medicines, cosmetics, and disinfectants. Peppermint is used for many different conditions and diseases, including atherosclerosis, angina pectoris, heart attack, cough, bronchitis, indigestion, stomach pains and spasms, colds, cholecystitis, chronic pancreatitis, cardiovascular diseases, depression, epilepsy, diarrhea, fatigue, muscle pain, physical trauma, and chronic colitis [36–38].

Probiotic bacteria, specifically lactobacilli and bifidobacteria, have gained significant attention in recent years for their potential in food preservation. Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer health benefits to the host. While their primary application is in promoting gut health, probiotic bacteria have also shown promise in extending the shelf life and improving the safety of various foods. Lactobacilli and bifidobacteria are commonly used probiotic strains due to their ability to survive and thrive in different food matrices [28,38]. These bacteria have been studied extensively for their antimicrobial properties, which are attributed to the production and accumulation of organic acids, bacteriocins, and hydrogen peroxide. These antimicrobial compounds inhibit the growth of spoilage microorganisms and foodborne pathogens, thereby preserving food quality and safety. Besides their antimicrobial activity, lactobacilli and bifidobacteria can also exhibit other functional properties that contribute to food preservation [28,39]. For example, they can produce exopolysaccharides, which enhance food product texture and stability. Probiotic bacteria can also exert antioxidant effects, reducing oxidative damage and extending the shelf life of foods. Various foods have been successfully preserved using probiotic bacteria. These include dairy products, fermented vegetables (like sauerkraut and kimchi), fermented meats, and even baked goods [40,41]. The incorporation of probiotic bacteria into these foods not only provides health benefits but also contributes to their preservation by inhibiting spoilage and pathogenic microorganisms. It is worth noting that the successful application of probiotic bacteria for food preservation depends on several factors, including the strain selection, viability during processing and storage, compatibility with the food matrix, and sensory acceptance by consumers [42]. Additionally, regulatory considerations and quality control measures need to be implemented to ensure the safety and efficacy of probiotic-containing foods. Overall, probiotic bacteria, particularly lactobacilli and bifidobacteria, offer promising potential as natural preservatives in a number of foods. Their ability to inhibit spoilage microorganisms, improve food safety, and provide health benefits makes them an attractive option for enhancing food shelf life and quality [40–42].

Lactobacillus helveticus 2/20 has a number of proven probiotic properties. Lactobacillus helveticus 2/20 has demonstrated high antimicrobial activity against *Salmonella abony*, *Escherichia coli*, *Staphylococcus aureus*, *Proteus vulgaris*, *Listeria monocytogenes*, and *Enterococcus faecalis*. It has exhibited resistance to many antibiotics applied in clinical practice, suggesting its inclusion in the composition of probiotics and further application in complex therapy. *Lactobacillus helveticus* 2/20 has demonstrated a high survival rate in model conditions of the gastrointestinal tract, retaining a high living-cell concentration (more than 10^{13} cfu/g). *Lactobacillus helveticus* 2/20 has allowed the conduction of batch processes, resulting in concentrates with a high viable cell content (10^{13} cfu/cm^3) [43].

The aim of the present study was to examine the chemical compositions and antimicrobial activities of lavender, peppermint, raspberry seed, and ylang-ylang EOs and their application in combination with probiotic *Lactobacillus helveticus* 2/20 cells (free or encapsulated cells) for chocolate mousse biopreservation. This is the first step towards researching hurdle technologies for the production of chocolate mousse without any chemical preservatives, and some of the highlights for such production are commented on in the conclusion.

2. Materials and Methods

2.1. Plant Materials

In the present study, the plant materials were purchased from "Pimenta Bulgaria" Ltd. (Sofia, Bulgaria) Lavender, ylang-ylang, raspberry seed, and peppermint EOs were obtained from the dried flowers of *Lavandula angustifolia* Mill. (0.0174 g EO/g DW) and of *Cananga odorata* (Lam.) Hook.f. & Thomson (0.0278 g EO/g DW), dried seeds of *Rubus idaeus* L. (0.0162 g EO/g DW), and dried leaves of *Mentha piperita* L. (0.0178 g EO/g DW). A total of 250 g of each plant material was weighed, ground, and passed through a 0.5 mm sieve. The moisture content analyzed using AOAC 934.06 (AOAC, 2007) was established to be 1.6% for lavender, 1.7% for raspberry seed, 1.6% for peppermint, and 1.8% for ylang-ylang [44].

2.2. Microorganisms

The *Lactobacillus helveticus* 2/20 probiotic strain was used in the experiments on the biopreservation of the chocolate mousse food emulsion. The strain is part of the cultural collection of the Department of Microbiology at UFT-Plovdiv.

Test microorganisms used for the examination of EO antimicrobial activity: *Salmonella abony* NTCC 6017, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* NBIMCC 1390, *Saccharomyces cerevisiae* ATCC 9763, *Bacillus subtilis* ATCC 19659, *Aspergillus niger* ATCC 1015, *Aspergillus flavus* ATCC 9643, *Penicillium chrysogenum* ATCC 28089, *Fusarium moniliforme* ATCC 38932.

2.3. Extraction of EOs

The contents of EOs were determined using water distillation in the laboratory glass apparatus of the British pharmacopoeia, modified by Balinova and Dyakov, % (v/w) [45].

2.4. Determination of Chemical Composition of EOs

The compositions of the EOs were determined using GC-MS in accordance with [28].

2.5. Determination of the Minimum Inhibitory Concentration (MIC) of EOs to Bacteria

The MIC of each EO against bacteria was determined using the CLSI method [46]. The EOs were subjected to serial two-fold dilutions in Mueller-Hinton broth (Merck, Germany) using a 96-well microtitration plate. Then, each well was inoculated with a microbial suspension at a concentration of 5×10^5 cfu/cm^3. After mixing, the plates were incubated at 30 °C for 24 h for the bacilli and at 37 °C for 18 h for the rest of the bacteria. After incubation, the optical density of the suspension was determined at λ = 600 nm (OD600). The MIC is the lowest concentration of EO which completely inhibits the test-microorganism growth.

2.6. Determination of the Minimum Inhibitory Concentration (MIC) of EOs to Yeasts

The MIC of each EO against bacilli and yeasts was determined using the CLSI method [47]. The EOs were subjected to serial two-fold dilutions in RPMI 1640 broth with glutamine, without bicarbonate and with an indicator (Sigma Aldrich, St. Louis, MO, USA), using a 96-well microtitration plate. Then, each well was inoculated with a microbial suspension at a concentration of 5×10^5 cfu/cm^3. After mixing, the plates were incubated at 30 °C for 24 h. After incubation, the optical density of the suspension was determined at λ = 600 nm (OD600). The MIC is the lowest EO concentration which completely inhibits the growth of the test microorganism.

2.7. Determination of the Minimum Inhibitory Concentration (MIC) of EOs to Filamentous fungi

The MIC for each EO was determined using the CLSI method [48]. The EOs were subjected to serial two-fold dilutions in RPMI 1640 broth with glutamine, without bicarbonate and with an indicator (Sigma Aldrich, USA), using a 96-well microtitration plate. A spore suspension at a concentration of 5×10^4 cfu/cm^3 was then inoculated into each well. After mixing, the plates were incubated at 30 °C for 48 h. After incubation, the plates were examined visually. The MIC is the lowest EO concentration which completely prevents any test-microorganism discernable growth.

2.8. Determination of the Antimicrobial Activity of EOs against Probiotic Strain Lactobacillus helveticus 2/20

The serial dilution method was used for determining the EO antimicrobial activity against the probiotic strain *Lactobacillus helveticus* 2/20 [9].

2.9. Encapsulation of Lactic Acid Bacteria in Emulsion

The encapsulation of lactic acid bacteria in emulsion was performed according to [28,49]. The encapsulated cells were then mixed with 1–2 cm^3 of saline solution and added to the respective chocolate mousse variants (defined below).

2.10. Preparation of the Chocolate Mousse Variants

The chocolate mousse (CM) was made from chocolate (52% cocoa content) and whipping cream. An amount of 400 g of chocolate was melted in a water bath at 65 °C, stirring periodically. Meanwhile, 600 cm^3 of cream was whipped using a mixer. The cooled chocolate (temperature around 45 °C) was added to the beaten cream in small portions with continuous stirring until the desired consistency was achieved. Then, the CM was divided into 50 g portions; there was one control sample, and the pre-selected EOs and/or free or encapsulated probiotic *Lactobacillus helveticus* 2/20 cells according to the following scheme were added to the rest of the portions:

Sample
Control (CM (50 g) without any EOs or any *Lactobacillus helveticus* 2/20 cells)
CM (50 g) + 0.25 cm^3 free *Lactobacillus helveticus* 2/20 cells
CM (50 g) + 0.25 cm^3 encapsulated *Lactobacillus helveticus* 2/20 cells
CM (50 g) + 0.25 cm^3 peppermint EO
CM (50 g) + 0.25 cm^3 peppermint EO + 0.25 cm^3 free *Lactobacillus helveticus* 2/20 cells
CM (50 g) + 0.25 cm^3 peppermint EO + 0.25 cm^3 encapsulated *Lactobacillus helveticus* 2/20 cells

The changes in the pH and the viable cell concentration as well as the specific microbiological indicators of the CM samples were monitored during refrigerated storage for 20 days.

2.11. Determination of Microbiological Indicators of Chocolate Mousse

- Mesophilic aerobic and facultative anaerobic bacteria, in accordance with ISO 4833:2004 [50];
- Filamentous fungi and yeasts in accordance with ISO 21527-2:2011 [51];
- *Escherichia coli* in accordance with ISO 16649-2:2001 [52];
- *Enterobacteriaceae* in accordance with ISO 21528-2:2017 [53];
- *Salmonella* sp. in accordance with ISO 6579:2003 [54];
- Coagulase-positive staphylococci in accordance with ISO 6888-1:2005 + A1:2005 [55].

2.12. Organoleptic Evaluation of the Chocolate Mousse Variants

The chocolate mousse variants were evaluated by 10 experts in the field of Food Science according to 6 parameters ranging from 0 (worst rating) to 9 (best rating): appearance, characteristic smell, sweetness, taste, aftertaste, and texture.

2.13. Statistical Analyses

Data from triplicate experiments were processed using MS Office Excel 2010 software version 14.0, using statistical functions to determine the standard deviation and the maximum evaluation error at levels of significance of $\acute{\alpha} < 0.05$.

3. Results and Discussion

3.1. Determination of the Chemical Compositions of Lavender, Raspberry Seed, Peppermint, and Ylang-Ylang Eos

The chemical compositions of lavender, peppermint, raspberry seed, and ylang-ylang EOs were determined (Tables 1–4).

Table 1. Chemical composition of lavender EO.

№	Compounds	RT	% Area
1	Camphene	4.09	0.07
2	β-Pinene	4.54	0.5
3	o-Cymene	5.35	0.26
4	Limonene	5.43	3.11
5	Eucalyptol	5.53	2.09
7	Linalool	6.86	35.43
8	3-Pinanol	7.34	0.25
9	Cyclopentanol, 3-isopropenyl-1,2-dimethyl-1-cyclopentanol	7.63	0.56
10	d-2-Bornanone	8.26	3.75

Table 1. *Cont.*

№	Compounds	RT	% Area
11	endo-Borneol	8.81	1.9
12	Terpinen-4-ol	8.96	3.08
13	Terpineol	9.33	0.4
14	Linalyl acetate	10.45	42.01
15	Lavandulol acetate	11.29	1.28
16	Isopulegol acetate	11.41	0.26
17	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate	13.78	0.3
18	Caryophyllene	15.01	0.6
19	cis-β-Farnesene	15.59	0.37
20	Germacrene D	16.41	0.21

RT—Retention Time; Area %—Peak area.

Table 2. Chemical composition of peppermint EO.

№	Compounds	RT	% Area
1	cis-3-Methylcyclohexanol	4.04	0.17
2	Camphene	4.09	0.13
3	3-Methylcyclohexanone	4.2	0.48
4	L-(1,2-Dimethyl-cyclopent-2-enyl)-ethanone	4.33	0.03
5	Sabinene	4.39	0.67
6	β-Pinene	4.54	3.80
7	2-Ethyl-1-hexanol	5.29	0.32
8	o-Cymene	5.35	0.42
9	D-Limonene	5.43	18.43
10	Eucalyptol	5.53	1.16
11	L-Menthone	8.37	12.55
12	Isomenthone	8.61	5.42
13	Levomenthol	8.67	3.39
14	Menthol	8.87	30.80
15	Isopulegol	8.96	1.18
16	Terpineol	9.33	0.27
17	cis-Dihydrocarvone	9.45	0.34
18	Carvone	10.64	16.33
19	Piperitone	10.9	0.50
20	Menthyl acetate	11.58	2.26
21	Caryophyllene	15.02	0.58

RT—Retention Time; Area %—Peak area.

Table 3. Chemical composition of ylang-ylang EO.

№	Compounds	RT	% Area
1	*p*-Methoxytoluene	5.32	1.13
2	Benzyl alcohol	5.62	2.11
3	Linalool	6.83	12.07
4	Methyl benzoate	7.36	12.59
5	Benzyl acetate	8.49	15.63
6	Citronellol	9.92	0.96
7	Geraniol	10.54	1.05
8	Eugenol	13.35	0.20
9	Geranyl acetate	13.77	3.42
10	Ylangene	13.83	1.26
11	Caryophyllene	15.01	16.08
12	Humulene	15.87	1.25
13	γ-Muurolene	16.23	0.15
14	Germacrene D	16.41	1.03
15	α-Farnesene	16.7	0.92
16	δ-Cadinene	17.09	0.81
17	Benzoic acid, 2-hydroxy-, 2-methylbutyl ester	17.43	1.44
18	Amyl salicylate	18.14	3.09
19	Diethyl Phthalate	18.39	17.72
20	cis-Isoeugenol	18.66	1.08
21	α-Cadinol	19.45	0.14
22	Cedryl acetate	20.81	1.34
23	Benzyl Benzoate	20.89	7.16
24	Phenylacetic acid, 2-methylphenyl ester	21.47	1.43
25	Benzoic acid, 2-hydroxy-, phenylmethyl ester	21.72	4.47

RT—Retention Time; Area %—Peak area.

Table 4. Chemical composition of raspberry EO.

№	Compounds	RT	% Area
1	Limonene	5.43	1
2	9-Octadecenal	22.31	14.23
3	Tetradecanal	22.42	25.13
4	Methyl linolelaidate	23.04	25.13
5	Pentadecanal	23.35	3.74

RT—Retention Time; Area %—Peak area.

Twenty compounds were identified in the lavender EO, eight of which were over 1.00% and the remaining twelve below 1.00%. Monoterpene and sesquiterpene hydrocarbons predominated. The main representatives of the monoterpene hydrocarbons were linalool (35.43%), linalyl acetate (42.01%), limonene (3.11%), lavender acetate (1.28%), terpin-4-ol (3.08%), and eucalyptol (2.09%); that of the sesquiterpene hydrocarbons was caryophyllene (0.06%) (Table 1).

Twenty-one components were identified in the peppermint EO, 10 of which were over 1.00% and the remaining 11 below 1.00%. The oil was dominated by monoterpene hydrocarbons and monoterpene oxygen derivatives. Monoterpene hydrocarbons were mainly represented by menthol (30.80%), D-limonene (18.43%), carvone (16.33%), L-menthone (12.55%), isomenthone (5.42%), levomenthol (3.39%), β-pinene (3.8%), methyl acetate (2.26%), and eucalyptol (1.16%) and the sesquiterpene hydrocarbons by caryophyllene (0.58%) (Table 2).

Twenty-five compounds were identified in the ylang-ylang EO, 19 of which were over 1.00% and the remaining less than 1.00% (Table 5). Monoterpene hydrocarbons and aromatic compounds predominated. The main representatives of the monoterpene hydrocarbons were linalool (12.07%) and geranyl acetate (3.42%). The aromatic compounds were represented by methyl benzoate (12.59%), benzyl acetate (15.63%), diethyl phthalate (17.72%), benzyl benzoate (7.16%), benzoic acid (4.47%), benzyl alcohol (2.11%), and amyl salicylate (3.09%) and the sesquiterion hydrocarbons by caryophyllene (16.8%) (Table 3). The results obtained were consistent with those of previous studies regarding ylang-ylang EO composition [34].

Table 5. Minimum inhibitory concentrations (MICs) of peppermint, lavender, raspberry seed, and ylang-ylang EOs on the growth of *Escherichia coli* ATCC 25922, *Salmonella abony* NTCC 6017, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* NBIMCC 1390, and *Enterococcus faecalis* ATCC 29212.

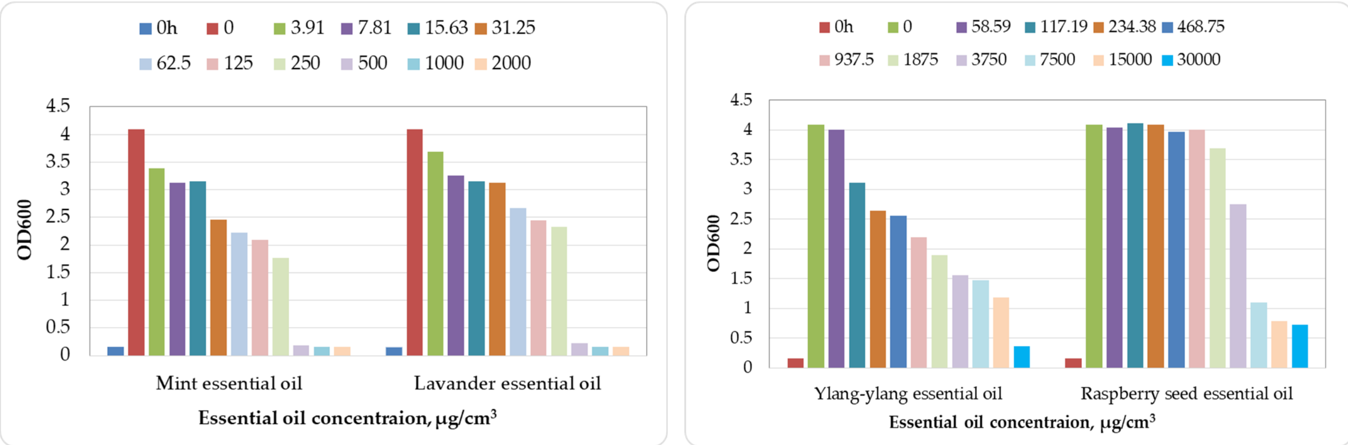
Test Microorganism	MIC, μg/cm^3			
	Peppermint EO	Lavender EO	Ylang-Ylang EO	Raspberry Seed EO
E. coli ATCC 25922	1000	500	>30,000	>30,000
S. abony NTCC 6017	500	15.63	>30,000	>30,000
S. aureus ATCC 25923	1000	2000	>30,000	>30,000
E. faecalis ATCC 29212	500	1000	>30,000	>30,000
P. aeruginosa NBIMCC 1390	500	500	>30,000	>30,000

Five compounds were identified in the raspberry seed EO, and all of them were above 1% (Table 4). The aroma of the raspberry seed EO was due to 9-octadecenal (14.23%), tetradecanal (25.13%), methyl linolelaidate (25.13%), pentadecanal (3.74%), and limonene (1%). The EO main components were monoterpene hydrocarbons and monoterpene oxygen derivatives. The results obtained were consistent with those of previous studies on raspberry seed, lavender, peppermint, and ylang-ylang EO compositions [31–33].

EOs are intricate combinations of volatile compounds extracted from many different plants. EO composition varies, primarily consisting of hydrocarbon terpenes and terpenoids [55]. Many active components of EOs and extracts exhibit antimicrobial and antioxidant activity.

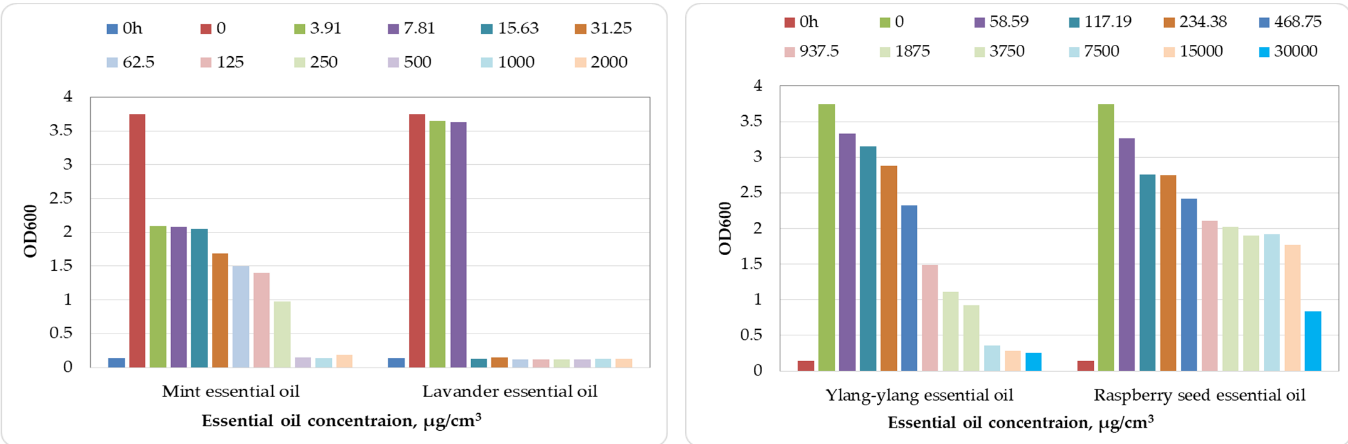
3.2. Determination of the Antimicrobial Activities of Lavender, Raspberry Seed, Peppermint, and Ylang-Ylang EOs against Pathogenic and Spoilage Microorganisms

The inhibitory activities of the EOs of peppermint, lavender, raspberry seed, and ylang-ylang against test pathogenic microorganisms causing food toxico-infections and intoxications were examined, and their corresponding minimum inhibitory concentrations (MICs) were determined. The results of the examination of the antimicrobial effects of EOs of peppermint, lavender, raspberry seed, and ylang-ylang against Gram-negative bacteria—*Escherichia coli* ATCC 25922, *Salmonella abony* NTCC 6017, *Pseudomonas aeruginosa* NBIMCC 1390—are given in Figures 1–3, respectively.



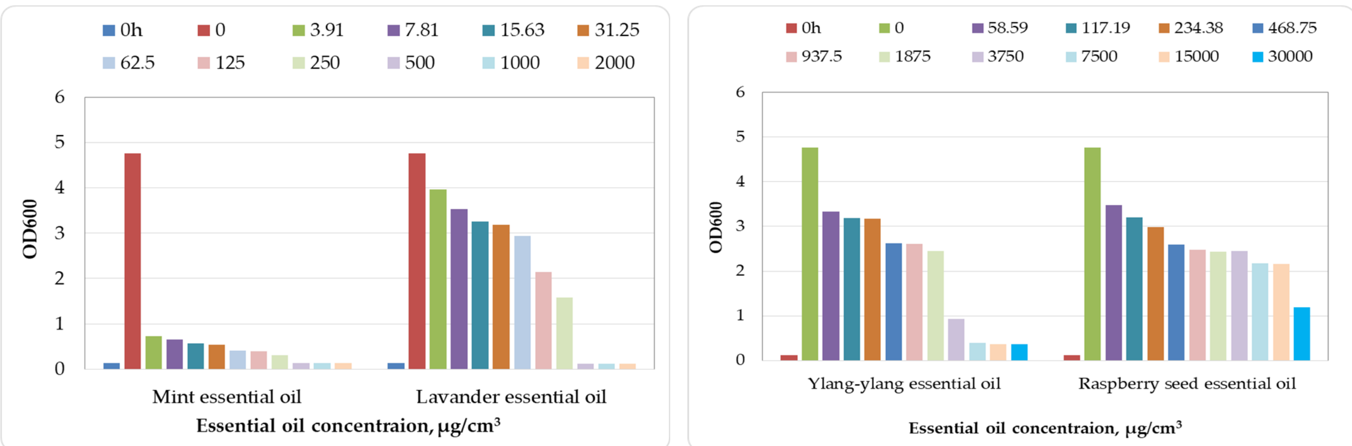
(**a**) (**b**)

Figure 1. Inhibitory activity of EOs on the growth of *Escherichia coli* ATCC 25922. (**a**) peppermint and lavender EOs. (**b**) raspberry seed and ylang-ylang EOs.



(**a**) (**b**)

Figure 2. Inhibitory activity of EOs on the growth of *Salmonella abony* NTCC 6017. (**a**) peppermint and lavender EOs. (**b**) raspberry seed and ylang-ylang EOs.



(**a**) (**b**)

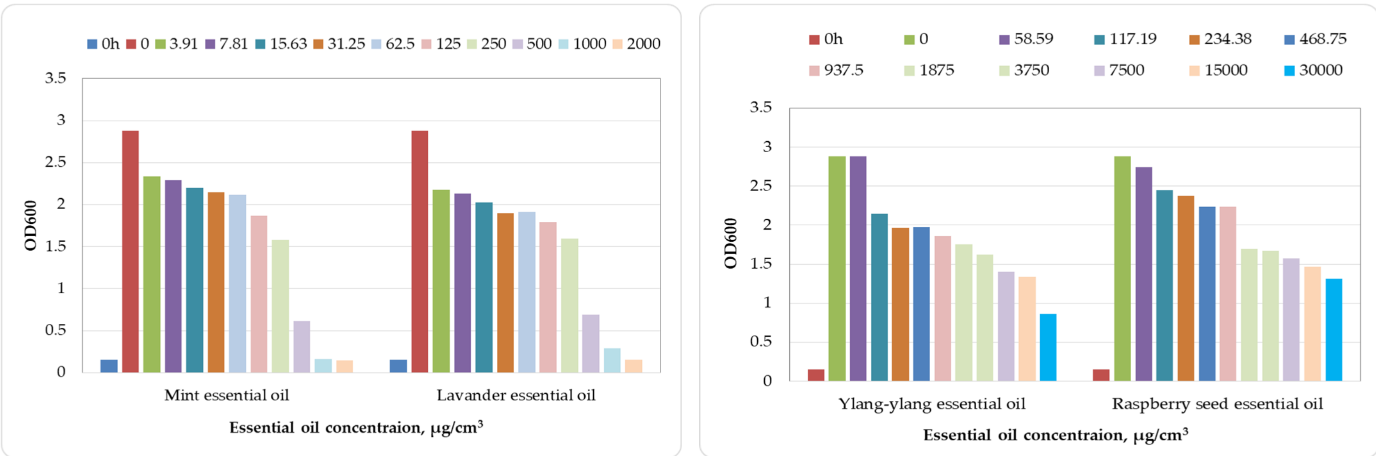
Figure 3. Inhibitory activity of EOs on the growth of *Pseudomonas aeruginosa* NBIMCC 1390. (**a**) peppermint and lavender EOs. (**b**) raspberry seed and ylang-ylang EOs.

The data shown in Figure 1a,b unequivocally indicated that EOs exhibited significant antimicrobial activity against *E. coli* ATCC 25922 and lavender EO had the highest inhibitory activity (MIC = 500 µg/cm^3), followed by peppermint EO (MIC = 1000 µg/cm^3). The antibacterial actions of raspberry seed and ylang-ylang EOs were significantly lower; the MICs were higher than 30,000 µg/cm^3 (Figure 1).

The obtained results indicated that the EOs of peppermint and lavender suppressed the growth of *Salmonella abony* NTCC 6017 better than *Escherichia coli* ATCC 25922. Similarly, the highest inhibitory activity (MIC = 15.63 µg/cm^3) was determined in lavender EO, followed by peppermint EO (MIC = 500 µg/cm^3) (Figure 2a). The antimicrobial action of the EOs of raspberry seed and ylang-ylang (MIC was higher than 30,000 µg/cm^3) on the growth of *Salmonella abony* NTCC 6017 (Figure 2b) was significantly lower. The results obtained are consistent with the research results obtained by Brnawi et al. (2019) [56].

Figure 3a,b indicate that the *Pseudomonas aeruginosa* NBIMCC 1390 cells were more sensitive to the action of EOs of peppermint and lavender, with the latter suppressing the growth of the pathogen to a high extent. The highest inhibitory activity was determined with peppermint EO and lavender EO (Figure 3a). The antimicrobial action of the EOs of raspberry seed and ylang-ylang (MIC was higher than 30,000 µg/cm^3) on the growth of *Pseudomonas aeruginosa* NBIMCC 13923 was significantly lower (Figure 3b).

The inhibitory activity of the peppermint, lavender, raspberry seed, and ylang-ylang EOs on the growth of Gram-positive bacteria—*Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212—was determined (Figures 4 and 5).

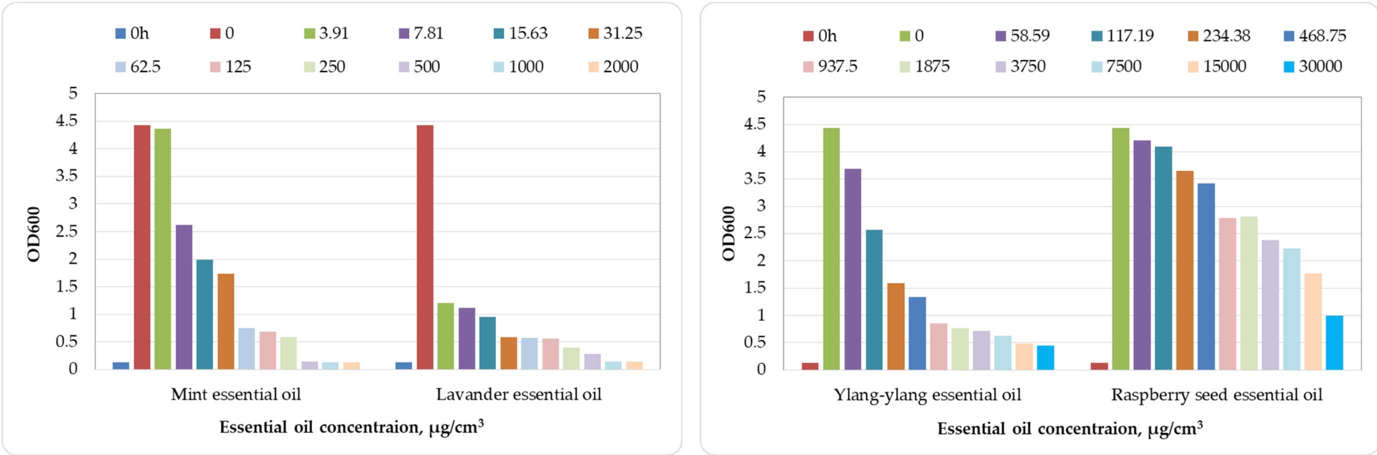


(**a**) (**b**)

Figure 4. Inhibitory activity of EOs on the growth of *Staphylococcus aureus* ATCC 25923. (**a**) peppermint and lavender EOs. (**b**) raspberry seed and ylang-ylang EOs.

The results reflected in Figure 4a,b indicate that peppermint and lavender EOs suppressed the growth of *Staphylococcus aureus* ATCC 25923. The highest inhibitory activity was determined in peppermint EO (MIC = 1000 µg/cm^3). The inhibitory effect of lavender EO on *Staphylococcus aureus* ATCC 25923 was lower (MIC = 2000 µg/cm^3) (Figure 4a). The antimicrobial action of the EOs of raspberry seed and ylang-ylang on the growth of *Staphylococcus aureus* ATCC 25923 was significantly lower (the MIC was more than 30,000 µg/cm^3) (Figure 4b).

The EOs demonstrated significant antimicrobial activity against the pathogenic microorganism *Enterococcus faecalis* ATCC 29212; the highest inhibitory activity was determined in peppermint EO (MIC = 500 µg/cm^3), followed by lavender EO (MIC = 1000 µg/cm^3) (Figure 5a). The antimicrobial action of raspberry seed and ylang-ylang EOs (MIC was higher than 30,000 µg/cm^3) on *Enterococcus faecalis* ATCC 29212 cells was significantly lower (Figure 5b).



(**a**) (**b**)

Figure 5. Inhibitory activity of EOs on the growth of *Enterococcus faecalis* ATCC 29212. (**a**) peppermint and lavender EOs. (**b**) raspberry seed and ylang-ylang EOs.

The minimum inhibitory concentrations (MICs) of the EOs of peppermint, lavender, raspberry seed, and ylang-ylang were determined based on the studies conducted to determine the antimicrobial activity of EOs on the growth of *Escherichia coli* ATCC 25922, *Salmonella abony* NTCC 6017, *Pseudomonas aeruginosa* NBIMCC 1390, and *Enterococcus faecalis* ATCC 29212 (Table 5).

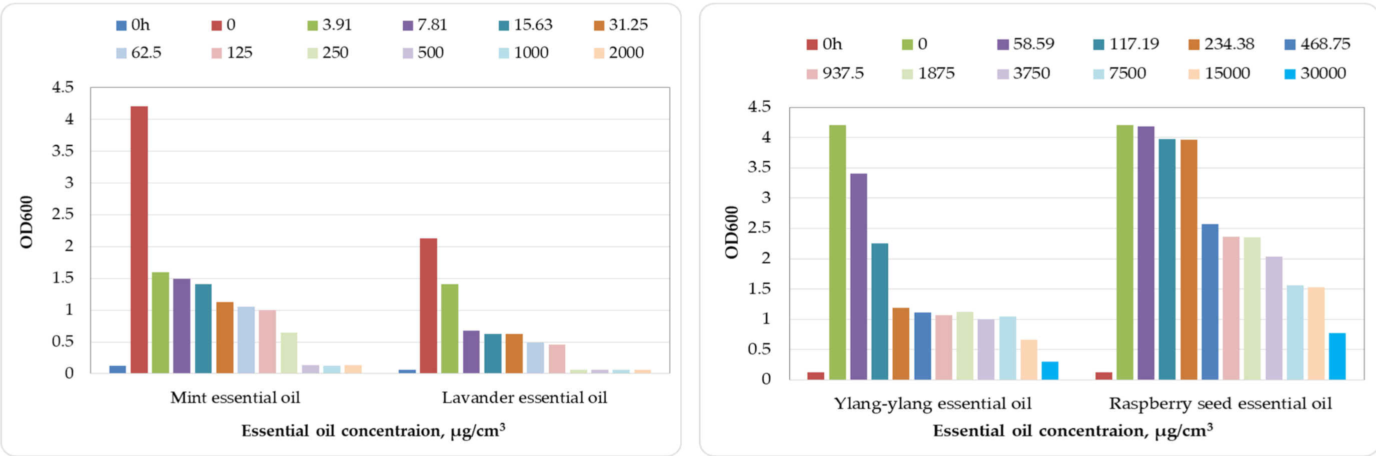
The lowest minimum inhibitory concentrations were those in peppermint EO (MIC = 500–1000 μg/cm^3) and lavender EO (MIC = 15.63–2000 μg/cm^3). The minimum inhibitory concentration (MIC) for raspberry seed EO and ylang-ylang EO (MIC being higher than 30,000 μg/cm^3) was too high. Gram-positive bacteria demonstrated greater sensitivity to the studied EOs, with MIC values in the 500–1000 μg/cm^3 range. The studied Gram-negative bacteria were less sensitive and the MICs were in the 15.63–2000 μg/cm^3 range. This was probably due to the difference in the cell wall composition of the two bacterial groups. The existence of an external membrane in the Gram-negative bacterial cell wall interferes with the diffusion of extracts through the membrane into the cell cytoplasm, which results in greater resistance of the cells to the EO action [57].

The EO action mechanism is attributed to their chemical composition and antibacterial action, which is not always the same [58]. The results obtained in the present research are consistent with the observations of [57] that EOs have a stronger antibacterial effect on Gram-positive bacteria (e.g., *Staphylococcus aureus*) than on Gram-negative bacteria (e.g., *Escherichia coli*). This is connected to the differences in the cell wall structure and composition of Gram-positive and Gram-negative bacteria. Obviously, the damage to the bacterial cellular membrane obtained is irreversible and the leakage of cytoplasmic components and ions and the lack of energy substrates lead to bacterial death [59–61].

The inhibitory activities of peppermint, lavender, raspberry seed, and ylang-ylang EOs on the growth of saprophytes that cause microbial spoilage have been studied, and the corresponding minimum inhibitory concentrations (MICs) have been determined. The observed antimicrobial effects of peppermint, lavender, raspberry seed, and ylang-ylang EOs on the growth of *Bacillus subtilis* ATCC 19659 are presented in Figure 6a,b.

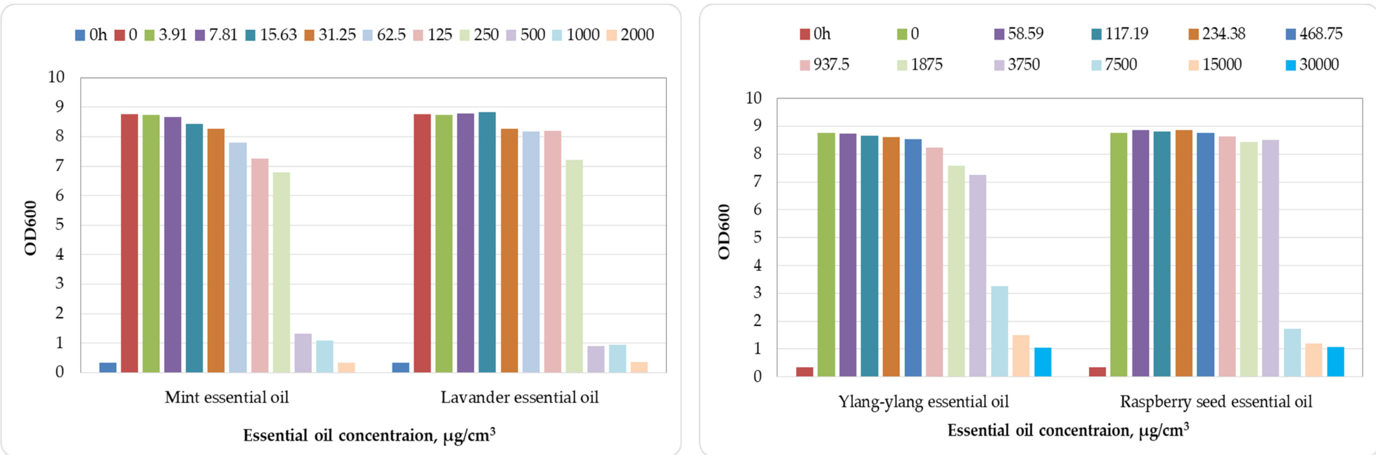
The data reflected in Figure 6a,b unambiguously indicate that EOs exhibit high antimicrobial activity against the saprophytic microorganisms; the highest inhibitory activity was determined in lavender EO (MIC = 250 μg/cm^3), followed by peppermint EO (500 μg/cm^3). The antimicrobial action of raspberry seed and ylang-ylang EOs (MIC exceeding 30,000 μg/cm^3) was significantly lower (Figure 6b).

The inhibitory activities of peppermint, lavender, raspberry seed, and ylang-ylang EOs on the growth of *Saccharomyces cerevisiae* ATCC 9763 were investigated (Figure 7a,b).



(a) (b)

Figure 6. Inhibitory activity of EOs on the growth of *Bacillus subtilis* ATCC 19659. (**a**) peppermint and lavender EOs. (**b**) raspberry seed and ylang-ylang EOs.



(a) (b)

Figure 7. Inhibitory activity of EOs on the growth of *Saccharomyces cerevisiae* ATCC 9763. (**a**) peppermint and lavender EOs. (**b**) raspberry seed and ylang-ylang EOs.

The results obtained show that EOs of peppermint and lavender suppressed the growth of *Saccharomyces cerevisiae* ATCC 9763 compared to spore-forming bacteria. The highest inhibitory activity was determined in peppermint EO and lavender EO (MIC = 2000 µg/cm^3) (Figure 7a). The antimicrobial action of EOs of raspberry seed and ylang-ylang (MIC being higher than 30,000 µg/cm^3) on the growth of *Saccharomyces cerevisiae* ATCC 9763 was significantly lower.

The inhibitory activity of peppermint EO, lavender EO, ylang-ylang EO, and raspberry seed EO on the growth of the fungal representatives *Penicillium chrysogenum* ATCC 28089, *Aspergillus niger* ATCC 1015, and *Aspergillus flavus* ATCC 9643 was determined. Peppermint EO exhibited high antimicrobial activity against the fungal strains *Penicillium chrysogenum* ATCC 28089, *Aspergillus niger* ATCC 1015, and *Aspergillus flavus* ATCC 9643 (MIC = 500 µg/cm^3). Lavender EO exhibited high antimicrobial activity against the growth of the fungal strains *Penicillium chrysogenum* ATCC 28089, *Aspergillus niger* ATCC 1015, and *Aspergillus flavus* ATCC 9643 (MIC = 500 µg/cm^3). Ylang-ylang EO suppressed the growth of the fungal strains *Penicillium chrysogenum* ATCC 28089, *Aspergillus niger* ATCC 1015, and *Aspergillus flavus* ATCC 9643 (MIC = 7500 µg/cm^3). The most sensitive were the spores of *Penicillium chrysogenum* ATCC 28089 (MIC = 3750 µg/cm^3) compared to other fungal representatives included in the present study. Raspberry seed EO exhibited less antimicrobial activity against the growth of the fungal strains *Penicillium chrysogenum* ATCC 28089, *Aspergillus niger* ATCC 1015, and *Aspergillus flavus* ATCC 9643 than the other EOs included in the present study.

The minimum inhibitory concentrations (MICs) of peppermint, lavender, raspberry seed, and ylang-ylang EOs against the growth of *Bacillus subtilis* ATCC 19659, *Saccharomyces cerevisiae* ATCC 9763, *Penicillium chrysogenum* ATCC 28089, *Aspergillus niger* ATCC 1015, and *Aspergillus flavus* ATCC 9643 were determined based on the studies conducted to determine the antimicrobial activity of the studied EOs (Table 6).

Table 6. Minimum inhibitory concentrations of peppermint, lavender, raspberry seed, and ylang-ylang EOs on the growth of *Bacillus subtilis* ATCC 19659, *Saccaromyces cerevisiae* ATCC 9763, *Penicillium chrysogenum* ATCC 28089, *Aspergillus niger* ATCC 1015, and *Aspergillus flavus* ATCC 9643.

Test Microorganism	**MIC, μg/cm^3**			
	Peppermint	**Lavender**	**Ylang-Ylang**	**Raspberry Seed**
Bacillus subtilis ATCC 19659	500	2000	≥30,000	≥30,000
Saccharomyces cerevisiae ATCC 9763	2000	2000	≥30,000	≥30,000
Asperillus niger ATCC 1015	500	500	7500	30,000
Aspergillus flavus ATCC+ 9643	500	500	3750	30,000
Penicillium chrysogenum ATCC 28089	500	500	7500	30,000

Grapefruit EO (MIC = 125–500 μg/cm^3) and lemon EO (MIC = 250–500 μg/cm^3), followed by peppermint EO (MIC = 500–2000 μg/cm^3) and lavender EO (MIC = 500–2000 μg/cm^3) had the lowest MICs. The MIC values for raspberry seed and ylang-ylang EOs were higher than 30,000 μg/cm^3.

The functional mechanism of EO antibacterial action is attributed to their chemical composition and antibacterial action, which are not always the same [57]. In many studies, the effect of EOs on the construction of the cell wall has been reported. The results we obtained are consistent with the Liang et al. (2011) [56] observation that EOs have stronger antibacterial effects on Gram-positive bacteria (for example, *Staphylococcus aureus*) than on Gram-negative bacteria (for example, *Escherichia coli*). This is attributed to the differences in the cell wall structure and composition of Gram-positive and Gram-negative bacteria. The irreversible damage to the bacterial cell membrane causes the leakage of cytoplasmic substances and ions and this, combined with the lack of energy substrates, leads to bacterial death. Due to the complex EO chemical composition, the mechanisms of their effects are also diverse, which makes it difficult to accurately determine the path of molecular action. It is also possible to suggest that each EO component has its own action mode [58–60].

Peppermint, lavender, raspberry seed, and ylang-ylang EOs exhibited suppression of the growth of pathogens causing gastrointestinal diseases and saprophytes. These results are important for food production, since, by introducing EOs, additional contamination with pathogens and saprophytes will be prevented and the growth of the accompanying microflora will be suppressed, which is important for food production.

3.3. Determination of the Resistance of Lactobacillus helveticus 2/20 to Different Concentrations of EOs of Lavender, Peppermint, Raspberry Seed, and Ylang-Ylang

The resistance of the probiotic strain *Lactobacillus helveticus* 2/20 to different concentrations of EOs of lavender, peppermint, raspberry seed, and ylang-ylang was also investigated. The experimental data indicate that the growth of *Lactobacillus helveticus* 2/20 was not affected by EOs at concentrations of up to 1%. This demonstrates that the selected probiotic strain can be introduced into food emulsions, together with the EOs of lavender, peppermint, raspberry seed, and ylang-ylang.

3.4. Biopreservation of Chocolate Mousse

Biopreservation is a method for preserving food quality and prolonging food shelf life via the introduction of substances of plant origin, animal origin, or microbial origin (metabolic substances with antimicrobial activity or living microorganisms (most commonly lactobacilli and bifidobacteria) that produce metabolites with high antimicrobial activity).

CM variants with free or encapsulated *Lactobacillus helveticus* 2/20 cells and peppermint EO were prepared. According to Bulgarian State Standards, the resulting CM variants should have a shelf life of 6 months in the frozen state and 3 weeks under refrigerated conditions (Figure 8a–f).

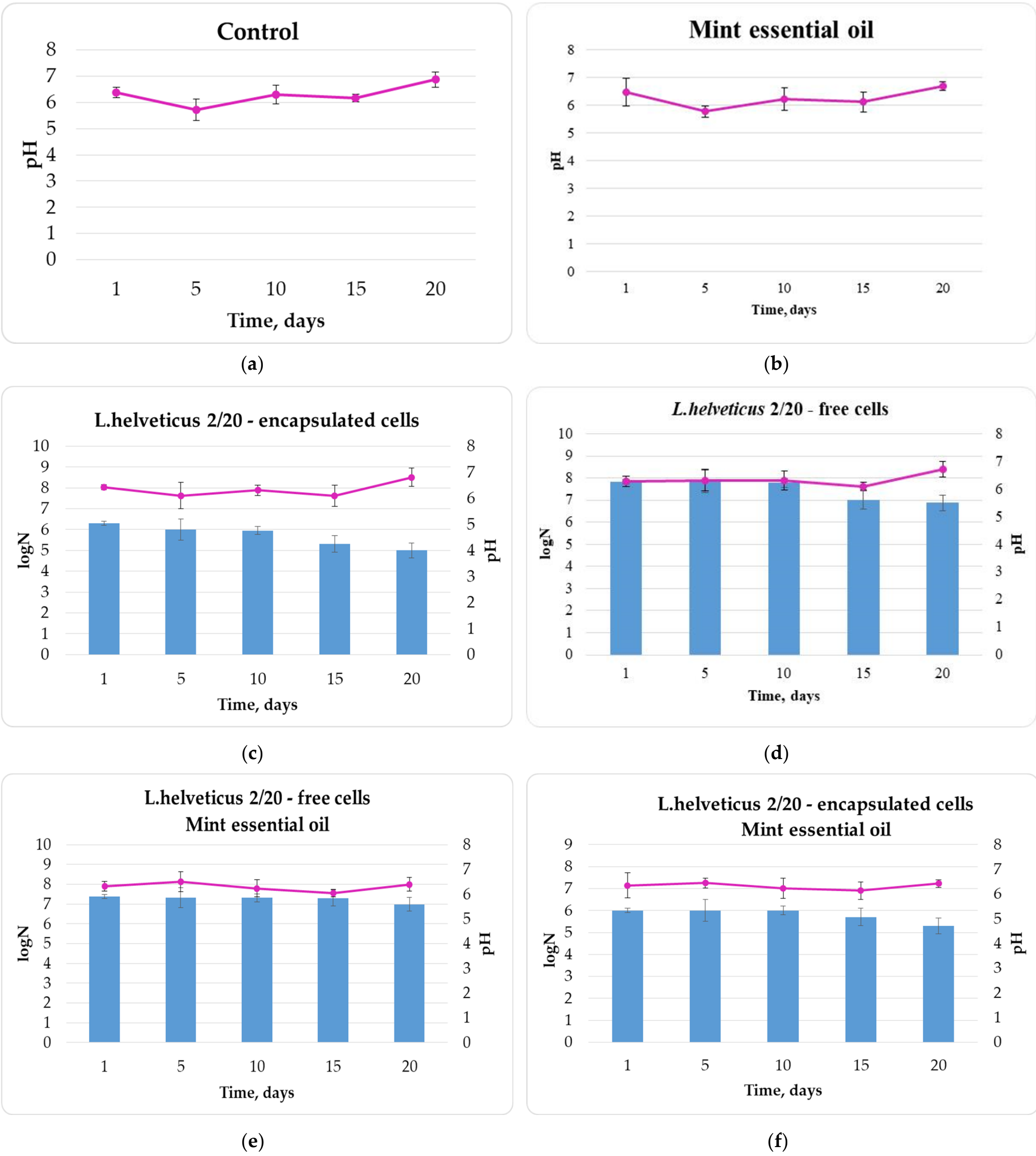


Figure 8. Biopreservation of CM. (**a**) control sample–pH. (**b**) biopreserved sample with peppermint EO–pH. (**c**) biopreserved with encapsulated lactobacilli cells sample–pH and viable cells. (**d**) biopreserved with free lactobacilli cells sample–pH and viable cells. (**e**) biopreserved with free lactobacilli cells and peppermint EO sample–pH and viable cells. (**f**) biopreserved with encapsulated lactobacilli cells and peppermint EO sample–pH and viable cells.

The changes in the pH and the microflora of the CM variants were monitored during the storage period of up to 20 days. The changes in the pH values during the storage process of the CM were slight (Figure 8a–f). The pH was maintained almost constant in the CM variants biopreserved with EOs, and the pH slightly decreased in the CM biopreserved with *Lactobacillus helveticus* 2/20 cells and EOs. Throughout the storage period, the probiotic strain retained a high concentration of viable cells (10^5 cfu/g) (Figure 8a–f).

With regard to pathogenic microorganisms, the CM variants met the standard requirements, and the number of active cells of mesophilic aerobic and facultatively anaerobic microorganisms, fungi, and yeasts was up to 10^2 cfu/g (Table 7). The number of viable *E. coli* cells in all CM variants from the 1st to the 20th day of storage was below 10 cfu/g. The number of viable *S. aureus* cells in all CM variants from the 1st to the 20th day of storage was below 100 cfu/g. No *Salmonella* sp. cells were found in any of the CM variants from the 1st to the 20th day of storage. The unwanted microflora in the CM variants biopreserved with lactobacilli and EO gradually decreased during the storage period, and no viable cells of pathogenic or saprophytic microorganisms after the 20th day were determined (Table 7). This could be explained by the demonstrated antimicrobial activity of the probiotic strain *Lactobacillus helveticus* 2/20 against mesophilic aerobic and facultatively anaerobic microorganisms, fungi, and yeasts, as well as the beneficial effect of peppermint, E.O.

Table 7. Microflora of biopreserved chocolate mousse variants during refrigerated storage (0 ± 4 °C). TBA–total bacterial abundance. FY–Fungi and yeasts. * according to the method of analysis (in accordance with ISO 21527-2:2011 [50]) the value of this parameter exceeded the limits set in the Standard, but the exact number of FY was not important.

	1st Day		**5th Day**		**10th Day**		**15th Day**		**20th Day**	
CM Variant	**TBA, cfu/g**	**FY, cfu/g**	**TBA, cfu/g**	**FY, cfu/g**	**TBA, cfu/g**	**FY, cfu/g**	**TBA, cfu/g**	**FY, cfu/g**	**TBA, cfu/g**	**FY, cfu/g**
CM control	20	<10	30	20	40	20	20	10	20	>10^5 *
CM with free *Lactobacillus helveticus* 2/20 cells	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
CM with encapsulated *Lactobacillus helveticus* 2/20 cells	<10	<10	10	<10	<10	<10	<10	<10	<10	<10
CM with peppermint EO	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
CM with peppermint EO and free *Lactobacillus helveticus* 2/20 cells	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
CM with peppermint EO and encapsulated *Lactobacillus helveticus* 2/20 cells	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10

The CM variants were evaluated according to six criteria: appearance, characteristic smell, sweetness, taste, aftertaste, and texture, with each criterion ranging from 0 (worst evaluation) to 9 (best evaluation) (Figure 9a–e).

It has been established that the different CM variants have good organoleptic indicators. The CM variants preserved with free or encapsulated *Lactobacillus helveticus* 2/20 cells and their combination with peppermint essential oil received the highest marks (Figure 9a–e).

The results obtained are consistent with those of Mutlu-Ingok et al. (2020) [62] and are of great importance for food emulsion production, since the introduction of EOs and probiotic bacteria would avoid additional contamination with pathogens and saprophytes [63]. Moreover, in addition to biopreservation, the intake of food enriched with probiotic bacteria ensures the entrance of a significant number of viable cells of probiotic microorganisms into the gastrointestinal tract.

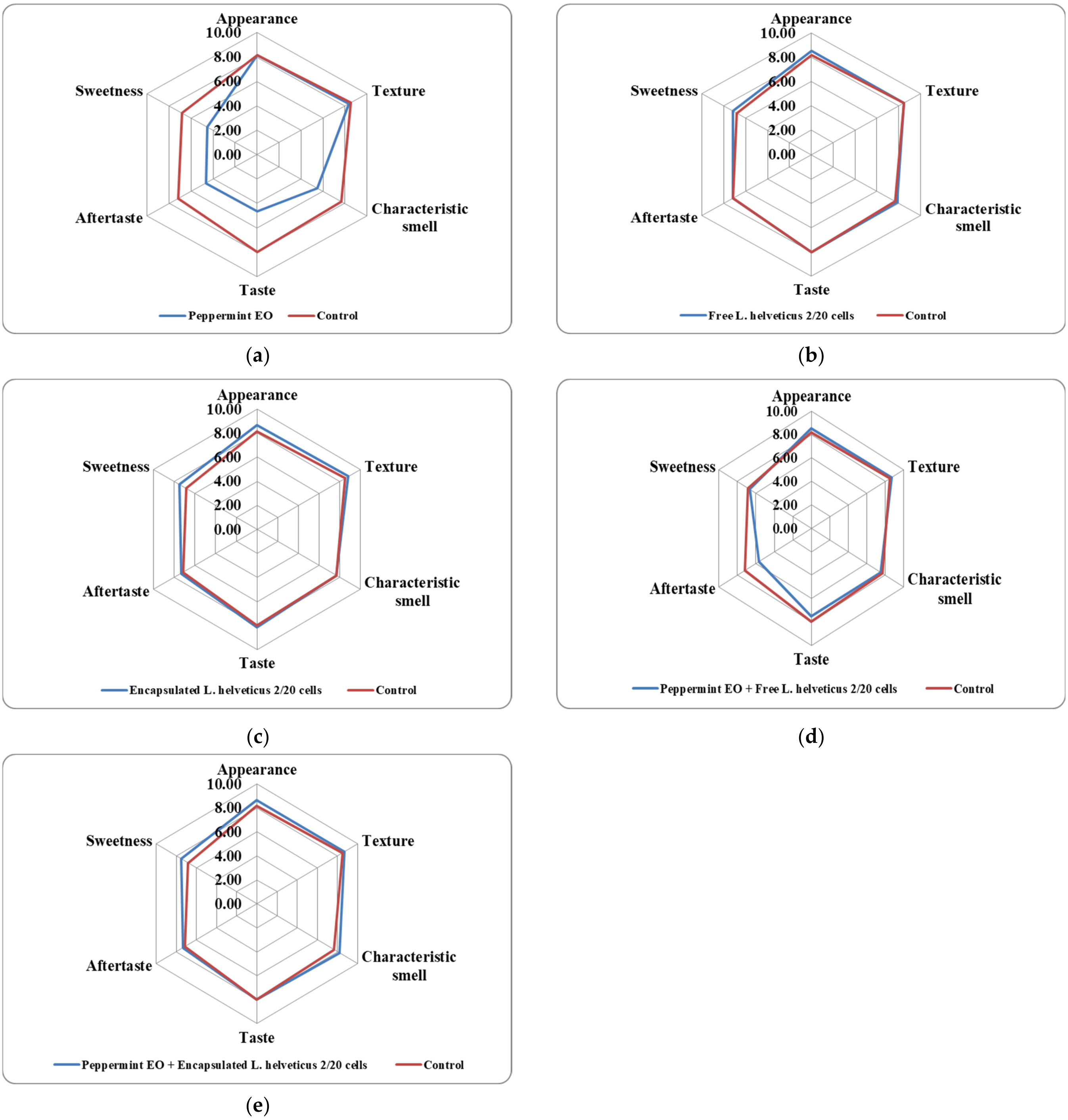


Figure 9. Organoleptic evaluation of the CM variants. (**a**) biopreserved with peppermint EO. (**b**) biopreserved with free lactobacilli cells. (**c**) biopreserved with encapsulated lactobacilli cells. (**d**) biopreserved with free lactobacilli cells and peppermint EO. (**e**) biopreserved with encapsulated lactobacilli cells and peppermint EO.

4. Conclusions

The component compositions of lavender, peppermint, raspberry seed, and ylang-ylang EOs have been determined. It has been shown that monoterpene and sesquiterpene hydrocarbons predominate in the EOs. The imported EO obtained from peppermint performs biological preservation of nutritional emulsions with preserved organoleptic characteristics and microbiological safety.

The introduction of free or encapsulated *Lactobacillus helveticus* 2/20 cells alone or jointly with peppermint EO provided biopreservation of the resulting food emulsions, retaining a high concentration of viable probiotic cells (10^6–10^7 cfu/g) under refrigerated storage process conditions for 20 days. The emulsions thus obtained can be considered as functional foods since they can be used to deliver probiotic *Lactobacillus helveticus* 2/20 cells to consumers.

The obtained results show that the combination of EOs and probiotic bacteria is suitable for applying hurdle technology principles with two biological hurdles. As a next stage of the research, we plan to expand our experiments by evaluating the individual and synergistic influences of these two biological hurdles via the monitoring of the kinetics of growth and development of individual microbial populations. Experiments related to the evaluation of the individual hurdles on the quality and functional properties of the final product are also planned.

Author Contributions: Conceptualization, Z.D., R.D.-K. and P.D.; methodology, R.D.-K.; software, G.K. and R.D.-K.; validation, G.K.; formal analysis, T.T., B.G., D.T., R.D.-K., D.B. and A.S.; investigation, T.T., B.G., D.T. and R.D.-K.; resources, Z.D.; data curation, G.K.; writing—original draft preparation, Z.D. and R.D.-K.; writing—review and editing, P.D., R.P. and G.K.; visualization, G.K.; supervision, Z.D.; project administration, G.K.; funding acquisition, G.K., P.D. and R.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Ministry of Education and Science of the Republic of Bulgaria under the project "Strengthening the research excellence and innovation capacity of University of Food Technologies—Plovdiv, through the sustainable development of tailor-made food systems with programmable properties", part of the European Scientific Networks National Programme funded by the Ministry of Education and Science of the Republic of Bulgaria (agreement № Д01-288/07.10.2020).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Alongi, M.; Anese, M. Re-thinking functional food development through a holistic approach. *J. Funct. Foods* **2021**, *81*, 104466. [CrossRef]
2. Martirosyan, D.M.; Singh, J. A new definition of functional food by FFC: What makes a new definition of functional food unique? *Funct. Foods Health Dis.* **2015**, *5*, 209–223. [CrossRef]
3. Akterian, S. Food Product Design. *Food Proc. Ind. Mag.* **2016**, *65*, 9–15. (In Bulgarian)
4. Singh, V.P. Recent approaches in food bio-preservation—A review. *Open Vet. J.* **2018**, *8*, 104–111. [CrossRef] [PubMed]
5. Putnik, P.; Pavlić, B.; Šojić, B.; Zavadlav, S.; Žuntar, I.; Kao, L.; Kitonić, D.; Kovačević, D. Innovative hurdle technologies for the preservation of functional fruit juices. *Foods* **2020**, *9*, 699. [CrossRef] [PubMed]
6. Leyva Salas, M.; Mounier, J.; Valence, F.; Coton, M.; Thierry, A.; Coton, E. Antifungal microbial agents for food biopreservation-a review. *Microorganisms* **2017**, *5*, 37. [CrossRef] [PubMed]
7. Ganguly, S. Basic principles for effective food preservation: A review. *Int. J. Pure Appl. Biosci.* **2013**, *1*, 84–85.
8. Souza, E.; Stamford, T.; Lima, E.; Trajano, V.; Filho, J. Antimicrobial effectiveness of spices: An approach for use in food conservation systems. *Braz. Arch. Biol. Technol.* **2005**, *48*, 549–558. [CrossRef]
9. Jirovetz, L.; Buchbauer, G.; Denkova, Z.; Slavchev, A.; Stoyanova, A.; Schmidt, E. Chemical composition, antimicrobial activities and odor descriptions of various *Salvia* sp. and *Thuja* sp. essential oils. *Ernahr. Nutr.* **2006**, *30*, 152–159.
10. Bakkali, F.; Averbeck, S.; Averbeck, D.; Idaomar, M. Biological effects of essential oils—A review. *Food Chem. Toxicol.* **2008**, *46*, 446–475. [CrossRef]
11. Tongnuanchan, P.; Benjakul, S. Essential Oils: Extraction, Bioactivities, and Their Uses for Food Preservation. *J. Food Sci.* **2014**, *79*, R1231–R1249. [CrossRef] [PubMed]
12. Chouhan, S.; Sharma, K.; Guleria, S. Antimicrobial Activity of Some Essential Oils Present Status and Future Perspectives. *Medicines* **2017**, *4*, 58. [CrossRef]
13. Winska, K.; Mączka, W.; Łyczko, J.; Grabarczyk, M.; Czubaszek, A.; Szumny, A. Essential Oils as Antimicrobial Agents-Myth or Real Alternative? *Molecules* **2019**, *24*, 2130. [CrossRef] [PubMed]

14. Yu, Z.; Tang, J.; Khare, T.; Kumar, V. The Alarming Antimicrobial Resistance in ESKAPEE Pathogens: Can Essential Oils Come to the Rescue? *Fitoterapia* **2020**, *140*, 104433. [CrossRef] [PubMed]
15. Burt, S.A. Essential oils: Their antibacterial properties and potential applications in foods: A review. *Inter. J. Food Microbiol.* **2004**, *94*, 223–253. [CrossRef]
16. Saban Kordali, S.; Kotan, R.; Mavi, A.; Cakir, A.; Ala, A.; Yildirim, A. Determination of the chemical composition and antioxidant activity of the essential oil of *Artemisia dracunculus* and of the antifungal and antibacterial activities of Turkish *Artemisia absinthium*, *A. dracunculus*, *Artemisia santonicum*, and *Artemisia* spicigera essential oils. *J. Agric. Food Chem.* **2005**, *53*, 9452–9458. [CrossRef]
17. Sylvestre, M.; Pichette, A.; Longtin, A.; Nagau, F.; Legaul, J. Essential oil analysis and anticancer activity of leaf essential oil of *Croton flavens* L. from Guadeloupe. *J. Ethnopharmacol.* **2006**, *103*, 99–102. [CrossRef]
18. Jirovetz, L.; Buchbauer, G.; Bail, S.; Denkova, Z.; Slavchev, A.; Stoyanova, A.; Schmidt, E.; Geissler, M. Antimicrobial Activities of Essential Oils of Mint and Peppermint as Well as Some of Their Main Compounds. *J. Essent. Oil Res.* **2009**, *21*, 363–366. [CrossRef]
19. Mulyaningsih, S.; Sporer, F.; Zimmermann, S.; Reichling, J.; Wink, M. Synergistic properties of the terpenoids aromadendrene and 1,8-Cineole from the essential oil of Eucalyptus Globulus against Antibiotic-Susceptible and Antibiotic-Resistant Pathogens. *Phytomedicine* **2010**, *17*, 1061–1066. [CrossRef]
20. Randrianarivelo, R.; Sarter, S.; Odoux, E.; Brat, P.; Lebrun, M.; Romestand, B.; Menut, C.; Andrianoelisoa, H.S.; Raherimandimby, M.; Danthu, P. Composition and antimicrobial activity of essential oils of *Cinnamosma fragrans*. *Food Chem.* **2009**, *114*, 680–684. [CrossRef]
21. Kazemi, S.M.; Rezaei, M. Antimicrobial effectiveness of gelatin-alginate film containing oregano essential oil for fish preservation. *J. Food Saf.* **2015**, *35*, 482–490. [CrossRef]
22. Calo, J.R.; Crandall, P.G.; O'Bryan, C.A.; Ricke, S.C. Essential oils as antimicrobials in food systems—A Review. *Food Control* **2015**, *54*, 111–119. [CrossRef]
23. Chellappandian, M.; Vasantha-Srinivasan, P.; Senthil-Nathan, S.; Karthi, S.; Thanigaivel, A.; Ponsankar, A.; Kalaivani, K.; Hunter, W.B. Botanical essential oils and uses as mosquitocides and repellents against dengue. *Environ. Int.* **2018**, *113*, 214–230. [CrossRef] [PubMed]
24. Rehman, A.; Jafari, S.M.; Aadil, R.M.; Assadpour, E.; Randhawa, M.A.; Mahmood, S. Development of active food packaging via incorporation of biopolymeric nanocarriers containing essential oils. *Trends Food Sci. Technol.* **2020**, *101*, 106–121. [CrossRef]
25. Tariq, S.; Wani, S.; Rasool, W.; Shafi, K.; Bhat, M.A.; Prabhakar, A.; Shalla, A.H.; Rather, M.A. A comprehensive review of the antibacterial, antifungal and antiviral potential of essential oils and their chemical constituents against drug-resistant microbial pathogens. *Microb. Pathog.* **2019**, *134*, 103580. [CrossRef] [PubMed]
26. Predoi, D.; Iconaru, S.L.; Buton, N.; Badea, M.L.; Marutescu, L. Antimicrobial activity of new materials based on lavender and basil essential oils and hydroxyapatite. *Nanomaterials* **2018**, *8*, 291. [CrossRef] [PubMed]
27. Cazella, L.N.; Glamoclija, J.; Soković, M.; Gonçalves, J.E.; Linde, G.A.; Colauto, N.B.; Gazim, Z.C. Antimicrobial activity of essential oil of *Baccharis dracunculifolia* DC (Asteraceae) Aerial Parts at Flowering Period. *Front. Plant Sci.* **2019**, *9*, 1–9. [CrossRef]
28. Teneva, D.; Denkova, Z.; Denkova-Kostova, R.; Goranov, B.; Kostov, G.; Slavchev, A.; Hristova-Ivanova, Y.; Uzunova, G.; Degraeve, P. Biological preservation of mayonnaise with *Lactobacillus plantarum* LBRZ12, dill, and basil essential oils. *Food Chem.* **2021**, *344*, 128707. [CrossRef] [PubMed]
29. Andrys, D.; Kulpa, D.; Grzeszczuk, M.; Bihun, M.; Dobrowolska, A. Antioxidant and antimicrobial activities of *Lavandula angustifolia* Mill. field-grown and propagated in vitro. *Folia Hortic.* **2017**, *29*, 161–180. [CrossRef]
30. Rehman, R.; Hanif, M.A.; Mushtaq, Z.; Al-Sadi, A.M. Biosynthesis of essential oils in aromatic plants: A review. *Food Rev. Int.* **2016**, *32*, 117–160. [CrossRef]
31. Ruberto, G.; Baratta, M.T. Antioxidant activity of selected essentialoil components in two lipid model systems. *Food Chem.* **2000**, *69*, 167–174. [CrossRef]
32. Adams, R.P. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy*; Allure: Carol Stream, IL, USA, 1995; p. 469. ISBN 978-0-9981557-2-2.
33. Kaiser, R.A.J. *Bioactive Volatile Compounds from Plants*; Teranishi, R., Buttery, R.G., Sugisawa, H., Eds.; American Chemical Society: Washington, DC, USA, 1993. [CrossRef]
34. Wei, A.; Shibamoto, T. Antioxidant/lipoxygenase inhibitory activities and chemical compositions of selected essential oils. *J. Agric. Food Chem.* **2010**, *58*, 7218–7225. [CrossRef] [PubMed]
35. Burdock, G.A.; Carabin, I.G. Safety assessment of Ylang-Ylang (*Cananga* spp.) as a food ingredient. *Food Chem. Toxicol.* **2008**, *46*, 433–445. [CrossRef] [PubMed]
36. Dorman, H.J.; Kosar, M.; Kahlos, K.; Holm, Y.; Hiltunen, R. Antioxidant prosperities and composition of aqueous extracts from *Mentha* species, hybrids, varieties and cultivars. *J. Agric. Food Chem.* **2003**, *51*, 4563–4569. [CrossRef] [PubMed]
37. Iscan, G.; Kirimer, N.; Kurkcuoglu, M.; Baser, K.N.; Demirci, F. Antimicrobial screening of *Mentha piperita* essential oils. *J. Agric. Food Chem.* **2002**, *50*, 3943–3946. [CrossRef] [PubMed]
38. Mahboubi, M.; Haghi, G. Antimicrobial activity and chemical composition of *Mentha pulegium* L. essential oil. *J. Ethnopharmacol.* **2008**, *119*, 325–327. [CrossRef]
39. Denkova-Kostova, R.; Teneva, D.; Tomova, T.; Goranov, B.; Denkova, Z.; Shopska, V.; Slavchev, A.; Hristova-Ivanova, Y. Chemical composition, antioxidant and antimicrobial activity of essential oils from tangerine (*Citrus reticulata* L.), grapefruit (*Citrus paradisi* L.), lemon (*Citrus lemon* L.) and cinnamon (*Cinnamomum zeylanicum* Blume). *Z. Naturforschung C* **2021**, *76*, 175–185. [CrossRef]

40. Udayakumar, S.; Rasika, D.M.D.; Priyashantha, H.; Vidanarachchi, J.K.; Ranadheera, C.S. Probiotics and beneficial Microorganisms in biopreservation of plant-based Foods and Beverages. *Appl. Sci.* **2022**, *12*, 11737. [CrossRef]
41. Chugh, B.; Kamal-Eldin, A. Bioactive compounds produced by probiotics in food products. *Curr. Opin. Food Sci.* **2020**, *32*, 76–82. [CrossRef]
42. More, A.S.; Ranadheera, C.S.; Fang, Z.; Warner, R.; Ajlouni, S. Biomarkers associated with quality and safety of fresh-cut produce. *Food Biosci.* **2020**, *34*, 100524. [CrossRef]
43. Denkova-Kostova, R.; Goranov, B.; Tomova, T.; Yanakieva, V.; Blazheva, D.; Denkova, Z.; Kostov, G. Investigation of probiotic properties of *Lactobacillus helveticus* 2/20 isolated from rose blossom of *Rosa damascena* Mill. *BIO Web Conf.* **2023**, *58*, 02002. [CrossRef]
44. AOAC International. *AOAC Official Method 934.06 for Moisture in Dried Fruits*; AOAC International: Gaithersburg, MD, USA, 2007.
45. Balinova-Tsvetkova, A.; Dyakov, G. Improved apparatus for microdistillation of rose flower. *Plant Sci.* **1974**, *11*, 79–85. (In Bulgarian)
46. Clinical and Laboratory Standards Institute. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically, Approved Standard*, 9th ed.; CLSI Document M02-A9; Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2012.
47. Clinical and Laboratory Standards Institute. *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts, Approved Standard*, 3rd ed.; CLSI Document M27-A3; Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2012.
48. Clinical and Laboratory Standards Institute. *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi; Approved Standard*, 2nd ed.; CLSI Document M38-A2; Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2012.
49. Bigdelian, E.; Razavi, S.H. Evaluation of survival rate and physicochemical properties of encapsulated bacteria in alginate and resistant starch in mayonnaise sauce. *J. Bioprocess. Biotech.* **2014**, *4*, 166. [CrossRef]
50. *ISO 4833:2003*; Microbiology of Food and Animal Feeding Stuffs—Horizontal Method for the Enumeration of Microorganisms—Colony-Count Technique at 30 Degrees C. ISO: Geneva, Switzerland, 2003.
51. *ISO 21527-2:2011*; Microbiology of Food and Animal Feeding Stuffs—Horizontal Method for the Enumeration of Yeasts and Moulds—Part 2: Colony Count Technique in Products with Water Activity Less than or Equal to 0.95. ISO: Geneva, Switzerland, 2011.
52. *ISO 16649-2:2001*; Microbiology of Food and Animal Feeding Stuffs—Horizontal Method for the Enumeration of Beta-Glucuronidase-Positive Escherichia coli—Part 2: Colony-Count Technique at 44 Degrees C Using 5-Bromo-4-Chloro-3-Indolyl Beta-D-Glucuronide. ISO: Geneva, Switzerland, 2011.
53. *ISO 21528-2:2017*; Microbiology of the Food Chain—Horizontal Method for the Detection and Enumeration of Enterobacteriaceae—Part 2: Colony-Count Technique. ISO: Geneva, Switzerland, 2017.
54. *ISO 6579:2002*; Microbiology of Food and Animal Feeding Stuffs—Horizontal Method for the Detection of Salmonella spp. ISO: Geneva, Switzerland, 2002.
55. *ISO 6888-1:1999/Amd 1:2003*; Microbiology of Food and Animal Feeding Stuffs—Horizontal Method for the Enumeration of Coagulase-Positive Staphylococci (Staphylococcus aureus and Other Species)—Part 1: Technique Using Baird-Parker Agar Medium—Amendment 1: Inclusion of Precision Data. ISO: Geneva, Switzerland, 2003.
56. Brnawi, W.I.; Hettiarachchy, N.S.; Horax, R.; Kumar-Phillips, G.; Ricke, S. Antimicrobial activity of leaf and bark cinnamon essential oils against *Listeria monocytogenes* and *Salmonella typhimurium* in broth system and on celery. *J. Food Process Preserv.* **2019**, *43*, e13888. [CrossRef]
57. Liang, L.F.; Yuan, H.; Li, C. In vitro antimicrobial effects and mechanism of action of selected plant essential oil combinations against four food-related microorganisms. *Food Res. Int.* **2011**, *44*, 3057–3064. [CrossRef]
58. Sawamura, M.; Onishi, Y.; Ikemoto, J.; Tu, N.T.M.; Phi, N.T.L. Characteristic odour components of bergamot (*Citrus bergamia* Risso) essential oil. *Flavour. Fragr. J.* **2006**, *21*, 609–615. [CrossRef]
59. Behbahani, B.A.; Noshad, M.; Falah, F. Cumin essential oil: Phytochemical analysis, antimicrobial activity and investigation of its mechanism of action through scanning electron microscopy. *Microb. Pathog.* **2019**, *136*, 103716. [CrossRef] [PubMed]
60. Behbahani, B.A.; Noshad, M.; Falah, F. Study of chemical structure, antimicrobial cytotoxic and mechanism of action of *Syzygium aromaticum* essential oil on foodborne pathogens. *Potravinarstvo Slovak J. Food Sci.* **2019**, *13*, 875–883. [CrossRef] [PubMed]
61. Noshad, M.; Hojjati, M.; Behbahani, B.A. Black Zira essential oil: Chemical compositions and antimicrobial activity against the growth of some pathogenic strain causing infection. *Microb. Pathog.* **2018**, *116*, 153–157. [CrossRef]
62. Mutlu-Ingok, A.; Devecioglu, D.; Dikmetas, D.N.; Karbancioglu-Guler, F.; Capanoglu, E. Antibacterial, antifungal, antimycotoxigenic, and antioxidant activities of essential oils: An updated review. *Molecules* **2020**, *25*, 4711. [CrossRef]
63. Vianna, T.C.; Marinho, C.O.; Marangoni Júnior, L.; Ibrahim, S.A.; Vieira, R.P. Essential oils as additives in active starch-based food packaging films: A review. *Int. J. Biol. Macromol.* **2021**, *182*, 1803–1819. [CrossRef] [PubMed]