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The Effect of Essential Oils on the Survival of *Bifidobacterium* in In Vitro Conditions and in Fermented Cream

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Abstract: Essential oils derived from plant materials are a mixture of compounds that exhibit antibacterial properties. Due to their distinct aroma, they also serve as a desirable natural additive for various food products, including dairy products. In this study, the essential oils of lemon peels, clove buds, and juniper berries were obtained by steam distillation and characterized using gas chromatography–mass spectrometry to determine their chemical compositions and effects on the viability of seven *Bifidobacterium* strains. Furthermore, the effect of essential oils on the viability of *Bifidobacterium animalis* subsp. *lactis* Bb-12 was investigated in cream samples during fermentation and after storage for 21 days at 6 °C. The fatty acid composition of fat extracted from essential oils containing sour cream samples and the volatile aroma compound profile of the sour cream samples were also determined chromatographically. Among the 120 compounds identified, monoterpene hydrocarbons were dominant in the essential oils of lemon peels (limonene and γ-terpinene) and juniper berries (sabinene and β-myrcene), while eugenol and eugenol acetate were abundant in the essential oil of clove buds. In addition to these compounds, butanoic and acetic acids were found in the tested sour cream samples. In turn, fat extracted from these samples was rich in saturated fatty acids, mainly palmitic acid. Among the tested strains of the genus *Bifidobacterium*, *B. animalis* subsp. *lactis* Bb-12 was the most sensitive to the essential oils of clove and juniper, as indicated by the larger growth inhibition zones. However, both the concentration and type of essential oils used had no effect on the number of cells of this strain present in the cream samples immediately after fermentation and after its 21-day storage, which suggests that the tested essential oils could be a natural additive to dairy products.

Keywords: clove buds; juniper berries; lemon peels; fatty acid composition; GC–MS; GC–TOF–MS; cream; fermentation

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1. Introduction

Bifidobacteria are known for their beneficial effects on human health, including their role in the proper functioning of the digestive tract [1]. In the large intestine, they hydrolyze sugars and produce lactic and acetic acids, which are metabolic end products. They reduce the pH of the contents in the large intestine and, thus, the levels of various harmful substances, including ammonia [2]. Bifidobacteria can also utilize ammonia as a source of nitrogen. Due to their low pH, they exert an antibacterial effect, inhibiting the growth of pathogenic microorganisms such as *Shigella*, *Salmonella*, and *Staphylococcus*, as well as enteropathogenic *Escherichia coli* strains. *Bifidobacterium* can reduce blood cholesterol and produce vitamins (mainly the B group), food enzymes, casein phosphatase, and

lysozyme [3]. Bifidobacteria-containing formulations used to treat constipation promote the production of organic acids by these bacteria, which stimulate normal intestinal peristalsis [4]. Studies also confirm that some of the bifidobacteria strains have excellent probiotic potential and, hence, can be included in formulas used for infants who cannot be fed with their mother's milk [5].

Bifidobacterium and *Lactobacillus acidophilus*, which have the ability to colonize and grow in the intestines, are the most commonly used microorganisms for the production of probiotic foods. Yogurts and yogurt drinks (containing live bacteria) are becoming increasing popular in the dairy market. An important aspect in the production of such products is ensuring that they have a high nutritional value, the right proportion of nutrients, and the benefits of additional vitamins, minerals, and other substances. Consumer awareness of nutrition has been growing in the past few years, posing challenges to both science and the food industry. Recent studies have been focusing on testing the possibility of replacing preservatives with natural substitutes, especially the plant-derived ones [6–9]. Essential oils extracted from plant materials are an ideal option. They are made up of organic compounds, such as esters, ethers, aldehydes, and ketones, which are characterized by a distinct aroma. Their biological activity results from the effects of individual dominant components or the synergistic action of a group of compounds. Some essential oils exhibit antimicrobial, antioxidant, and anticancer activities, while others have sedative, choleretic, and stimulatory effects on gastric function and secretion of digestive juices [10–19].

Besides enhancing the organoleptic characteristics, essential oils added in food products can promote their microbiological stabilization. They are often used as a flavoring agent for meat or meat-based products. The available literature describes the biological properties of essential oils extracted from clove buds, citrus peels, and juniper berries [10,12–15,17,18,20]. However, there are no reports on the effects of essential oils obtained from the above substances on *Bifidobacterium* bacteria, which is used as a component in probiotic fermented dairy products.

Therefore, this study aimed to investigate the effect of the essential oils extracted from clove buds, lemon peels, and juniper berries on the viability of selected *Bifidobacterium* strains in in vitro conditions. In addition, the effect of the concentration of these essential oils on the viability of the most sensitive *Bifidobacterium* strain was investigated in cream samples during their fermentation, and after storage, at 6 °C for 21 days. If there was no influence of the tested essential oils and their concentrations on the viability of the most sensitive strain (*B. animalis* subsp. *lactis* Bb-12) in the cream, we assumed that the other tested strains would not show any sensitivity to the tested essential oils added to the fermented cream. The chemical composition of the essential oils used was analyzed using gas chromatography–mass spectrometry (GC–MS). The fatty acid composition of the fat extracted from the sour cream samples was also determined chromatographically after its storage for 21 days. The volatile aroma compound profiles of sour cream samples enriched with essential oils were determined by the solid-phase microextraction (SPME)-GC–time-of-flight (TOF)-MS method.

2. Materials and Methods

2.1. Materials and Chemicals

Dried juniper berries (Species: *Juniperus communis* L.; Genus: *Juniperus* L.; Family: Cupressaceae; Order: Pinales; Class: Pinopsida; Division: Coniferophyta), clove buds (Species: *Syzygium aromaticum* L.; Genus: *Syzgium*; Family: Myrtaceae; Order: Myrtales; Class: Magnoliopsida; Division: Magnoliophyta), and fresh lemons (Species: *Citrus limon* L.; Genus: *Citrus* L.; Family: Rutaceae; Order: Sapindales; Class: Magnoliopsida; Division: Magnoliophyta) used in the study were purchased from a local market (Warsaw, Poland) in December 2017. All the solvents (methanol, dichloromethane, chloroform, and dimethyl sulfoxide (DMSO), *n*-hexane) and reagents (potassium chloride, anhydrous magnesium sulfate, sodium hydroxide, and boron trifluoride) used were of analytical grade and purchased from Avantor Performance Materials Poland (Gliwice, Poland). The fatty acid methyl esters

(FAME) reference standard (certified) mixture (consisting of 37 fatty acids ranging from C4 to C24) was obtained from Supelco (Bellefonte, PA, USA).

The *Bifidobacterium* strains used in this study (*B. animalis* subsp. *lactis* Bb-12, *B. bifidum* 89, *B. infantis* 45/03, *B. lactis* AD 600, *B. lactis* HN 019, *B. longum* AD 50, and *B. longum* KNA 1/08) were supplied by the Museum of Clean Cultures of the Division of Milk Technology of Warsaw University of Life Sciences (Poland).

2.2. Essential Oil Preparation

The lemons were washed carefully using tap water, and peels were removed and cut into pieces using a knife. Then, the pieces (40 g) were steam-distilled in a 1000 mL round-bottomed flask for 6 h. The distillate was cooled to the ambient temperature (20 ± 2 °C) and dried using anhydrous magnesium sulfate to remove the remaining water. The obtained essential oil was stored in a dark glass bottle at −21 °C until further analyses. The essential oils of juniper berries and clove buds were isolated using the same procedure, but in this case the dry plant material was ground in a blender before steam distillation (20 g of plant material and 500 mL of distilled water). The yield of essential oils was calculated using the formula: yield oil (%) = weight of oil (g)/weight of sample (g) × 100%.

2.3. The GC–MS Analysis of Essential Oils

The chemical components of lemon peel, clove bud, and juniper berry oils were determined using a Hewlett-Packard HP 7890A gas chromatograph instrument coupled with a 5975C mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) with an SLB-5MS (25 m × 0.2 mm × 0.33 μm) column. The samples of essential oils were directly injected onto a gas chromatograph. Helium gas was used as a carrier at a constant flow rate of 0.8 mL/min. The initial oven temperature was set at 40 °C for 1 min, and then the temperature was raised to 180 °C at a rate of 6 °C/min and further to 280 °C at 20 °C/min. The mass spectra were recorded in the electron impact mode (70 eV) at a scan range of m/z 33–350. The identification of volatile compounds was carried out based on the comparison of their retention indices (RI) and mass spectra with appropriate standards. If standards were not available, tentative identification was performed by comparing the mass spectra of the compounds with the spectral data from NIST 05, and their RI with the literature data. The RI of each compound was calculated using a homologous series of C6–C16 *n*-alkanes. Obtained results were calculated as a percentage composition of identified compounds.

2.4. The Effects of Essential Oils on the Viability of Bifidobacterium in In Vitro Conditions

The bacterial strains were cultured overnight in a BSM broth (Merck KGaA, Darmstadt, Germany) before their inoculation. The antibacterial activity of the obtained essential oils was evaluated using the well diffusion method [21]. Briefly, the bacterial suspension was distributed into the BSM agar medium at a density of 6–7 log(CFU/mL) and then poured into a Petri dish. After the medium had solidified, 5-mm wells were cut into it and 20 μL of each essential oil was applied at a concentration of 100%, 75%, 50%, 25%, and 0%. Each of the tested essential oils were dissolved in a mixture of three organic solvents in a 4:1:1 volume ratio (chloroform:methanol:DMSO). The solvent mixture was used as the control sample (0%). After adding the essential oils, the Petri dishes with wells were incubated at 37 °C for 72 h under anaerobic conditions (Anaerocult A, Merck, Germany) in an anaerobic jar (Merck, Germany) and then the zone of growth inhibition was measured. The experiments were repeated twice, and two parallel results were obtained each time.

2.5. The Effect of the Essential Oil Concentration on the Viability of B. animalis Subsp. lactis Bb-12 in Cream during Fermentation

Ultraheat-treated cream "Łowicka tortowa" (OSM Łowicz, Łowicz, Poland), with a fat content of 36%, was used in the experiments. Each tested essential oil, at a concentration of 100.0, 50.0, 25.0, 12.5, and 0.0 μg, was added to an 100-mL portion of cream followed by a culture of *B. animalis* subsp. *lactis* Bb-12 that was added at a density of 8.2 ± 0.4 log(CFU/mL)

overnight. The samples were fermented at 37 °C for 5 h and were then stored at 6 °C for 21 days. Before and after incubation, as well as after the 21 days of storage, the bifidobacteria population was determined using the deep plate method with the BSM agar as a culture medium. The Petri dishes were incubated at 37 °C for 72 h under anaerobic conditions (Anaerocult A, Merck, Germany) in an anaerobic jar. After incubation, all characteristic bacterial colonies grown were counted and expressed as log(CFU/mL). The experiments were repeated twice, and two parallel results were obtained each time.

2.6. Fat Extraction

Sour cream samples (9 g) were weighed into a 50-mL Eppendorf conical tube and a mixture of two solvents, chloroform and methanol (2:1; *v*/*v*; 30 mL), were added. The samples were slowly mixed in a vortex apparatus for 5 s and were then centrifuged at 6170× *g* (Biofuge Stratos, Thermo Fisher Scientific, Waltham, MA, USA) for 5 min. After centrifugation, the bottom layer was transferred into a separatory funnel using a Pasteur pipette and a 0.9% KCl solution (30 mL) was added. The resulting mixture was shaken gently and left aside for separation into two phases. The lower phase was collected and dried by adding a pinch of anhydrous magnesium sulfate. After the drying agent was filtered off through a Whatman No. 1 paper filter, the remaining solvent was evaporated under reduced pressure in a rotary evaporator (Rotavapor R-215, Büchi Labortechnik, Switzerland) at 40 °C. The extracted fat samples were weighed, flushed with nitrogen, and stored at −21 °C until further use. The percentage of total extractable fat was calculated.

2.7. Fatty Acid Analyses

The fatty acid compositions of the fat extracted from the sour cream samples were analyzed using a Trace 1300 gas chromatograph equipped with an SP-2560 capillary column (100 m × 0.25 mm × 0.2 µm; Supelco, Darmstadt, Germany) and a flame ionization detector after its derivatization to FAME, according to the AOCS Official Method Ce 1k-07 [22]. Hydrogen was used as the carrier gas at a flow rate of 1.5 mL/min. Initially, the oven temperature was set at 160 °C and was then increased at 12 °C/min to 220 °C, which was maintained for 20 min. The temperatures of the injector and detector were set at 240 °C. Fatty acids were identified by comparing their retention times with those of certified FAME. Their content was estimated in a percentage using the AOCS Official Method Ce 1h-05 (2005) [23].

2.8. Analyses of Volatiles Extracted from Cream and Sour Cream Samples

Volatiles were extracted by SPME using a carboxene/polydimethylsiloxane fiber (Supelco, Bellefonte, PA, USA) mounted in a CTC CombiPal autosampler (Agilent Technologies, Santa Clara, CA, USA). For the analysis of each volatile, 5 g of a sample was placed into 20-mL vials, spiked with 1 µg of an internal standard (naphthalene d8), and sealed with polytetrafluoroethylene/silicon septa caps. To avoid the influence of temperature on the formation of volatiles, the compounds were extracted without additional heating from the headspace at a stable room temperature of 21 °C for 30 min. The identification of the compounds was performed using a GCxGC–TOF-MS system with a ZOEX cryogenic (N2) modulator (Pegasus IV, LECO, St. Joseph, IL, USA) which was equipped with an SLB-5 MS (30 m × 0.25 mm × 0.5 µm) column (Supelco Bellefonte, PA, USA) coupled to a Supelcowax-10 (1.1 m × 0.2 mm × 0.2 µm) column (Supelco Bellefonte, PA, USA) and operated in a multidimensional chromatography mode. The temperature of the injector was set to 250 °C. During the injection, the fiber was exposed for 5 min in the splitless mode (1-min purge time). The conditions used for chromatography were as follows: helium flow, 0.8 mL/min; initial oven temperature, 40 °C (for 1 min) which was then increased to 180 °C at 6 °C/min and then further to 250 °C at 20 °C/min. For the two-dimensional analysis, the modulation time was optimized and set to 4 s and mass spectra were collected at a rate of 150 spectra/s. The transfer line was kept at 280 °C and the ion source was heated to 250 °C. Volatiles were identified by comparing the RI and the mass spectra of the eluting

compounds with those of the NIST 05 library match. A mixture of *n*-alkanes (C6–C20), dissolved in pentane which was obtained from Supelco (Bellefonte, PA, USA), was used for determining the RI of the volatiles. Calculations were carried out using Chroma TOF software (version 3.34). The semiquantification of compounds, which is represented by the average relative peak area of three replicates (arbitrary units presented as the mean value of three determinations), was achieved using the characteristic ions listed in Table 4. The results do not correspond to the absolute amount of a compound present in the samples, but they were calculated and used only for evaluating the differences between the analyzed samples.

2.9. Statistical Analyses

All the results were reported as an arithmetic average and the standard deviation was calculated from the replicates. Statistical analyses were performed using the Statgraphics Plus 4.0 package (Statgraphics Technologies, Inc., The Plains, VA, USA). The differences between the mean scores were determined using the analysis of variance (ANOVA). The differences between the means of multiple groups were analyzed using a two- or three-way ANOVA with Tukey's multiple range tests ($\alpha = 0.05$, $p < 0.05$).

3. Results and Discussion

3.1. Essential Oil Composition

The chemical compositions of the essential oils obtained from lemon peels, clove buds, and juniper berries by steam distillation are presented in Table 1. The GC–MS analysis indicated that the essential oils obtained were a mixture of various compounds, which mainly included mono- and sesquiterpene hydrocarbons and their oxygenated derivatives. Among the 120 identified components representing 96.59–99.54% of the total oils, 34, 47, and 72 belonged to the etheric oil obtained from clove buds, lemon peels, and juniper berries, respectively. Monoterpene hydrocarbons (68.53%) and oxygenated monoterpenes (24.78%) were the dominant group in the oil samples from lemon peels. However, the content of sesquiterpene hydrocarbons in these samples was relatively low (4.09%). The essential oil from juniper berries also had abundant monoterpenes (hydrocarbons and oxygenated monoterpenes) which accounted for 54.66% of the total. Sesquiterpenes (38.25%) and several nonterpene components, such as 2,3-hexanedione and hydroxymethyl 2-hydroxy-2-methylpropionate, were also identified in the studied oils. The main components present in the monoterpene hydrocarbon fraction of the essential oil from juniper berries were β-myrcene (6.45%), sabinene (6.19%), α-pinene (5.71%), limonene (4.78%), p-cymene (3.98%), and β-pinene (3.59%), while α-thujene (3.12%), α-terpinene (1.61%), and γ-terpinene (1.81%) were found in lower amounts. Among the oxygen-containing monoterpenes identified in the essential oil from juniper berries, linalool, α-campholenal, (E)-pinocarveol, α-phellandren-8-ol, p-cymen-8-ol, and verbenone were dominant. In turn, the sesquiterpene hydrocarbon fraction found in this essential oil consisted of α-cubebene (1.28%), β-elemene (2.01%), caryophyllene (1.54%), α-farnesene (1.67%), humulene (3.68%), α-muurolene (1.26%), germacerene D (1.54%), β-cubebene (2.88%), γ-amorphene (1.17%), γ-cadinene (1.74%), δ-cadinene (3.50%), and germacrene B (1.76%). A study showed that a total of 70 compounds were identified in the essential oils obtained from fresh juniper berries harvested from May 2010 to January 2011 [24]. Depending on the period of harvest of juniper fruits, monoterpene hydrocarbons accounted for 18.91–45.26% of the total volatiles of the obtained essential oils. The juniper berries collected in May had a similar content of monoterpene hydrocarbons as determined in the presented study, but they differed in the amount of the main representatives of this class of compounds. Compared to the content determined in the present study, α-pinene was at a higher level but sabinene and β-myrcene were identified in lower amounts. Moreover, monoterpene hydrocarbons were predominant in the essential oils isolated by steam distillation from ripe juniper berries collected from both the southwestern and central north regions of the Republic of Macedonia [25]. The main components of the monoterpene hydrocarbon fraction were α-

and β-pinene, β-myrcene, sabinene, and limonene. The content of compounds also differed the most in the oils obtained from the Macedonian juniper berries. Two Macedonian samples contained similar levels of β-pinene and sabinene, as determined in our study, whereas the amount of α-pinene was higher and limonene was slightly lower. The differences in the chemical composition of juniper oils, as well as in the amount of individual components, may be related to the geographical origin of the plant, the degree of ripeness of the berries, and the age of the berries used in the steam distillation [26]. The essential oils obtained from ripe and unripe wild juniper fruits in the Molise region were rich in α-pinene [24]. However, it has been reported that the amount of this compound may decrease as the fruit ripens.

The chemical compositions of the essential oils obtained from citrus peels, especially the content of monoterpene hydrocarbons and oxygenated monoterpenes, may also change during fruit ripening. Among the four types of citrus (bitter orange, orange maltaise, mandarin, and lemon), the highest level of limonene, which is one of the most dominant monoterpene hydrocarbons, was already reached at the immature fruit stage [27]. These observations could be useful to obtain essential oils with a high yield and with a high content of limonene. Our results also showed that limonene (31.51%) was the major volatile compound in yellow lemon peels. Furthermore, limonene (37.94%) and β-pinene (25.44%) were also dominant components in Tunisian essential oils isolated from lemon samples [28]. These active ingredients are often used for preparing perfumes, medicines, and flavoring agents, and their presence can have an impact on the antioxidant and antibacterial properties of essential oils. The essential oil of the Meyer lemon was found to exhibit the highest activity against *Bacillus cereus,* and that of the Interdonato lemon showed the highest activity against *E. coli* (the diameter of the inhibition zone: 15 mm) [29]. Other identified volatile components in the monoterpene hydrocarbon fraction included γ-terpinene (12.53%), α-pinene (2.68%), sabinene (3.06%), β-pinene (7.14%), β-myrcene (4.49%), and p-cymene (4.06%). The oxygenated monoterpene fraction included terpinolene (1.06%), linalool (2.76%), terpinene-4-ol (2.06%), α-terpineol (3.04%), neral (2.74%), geranial (4.05%), geraniol (3.38%), neryl acetate (1.81%), and geranyl acetate (1.84%). Sesquiterpenes were the third group of chemical compounds identified in the essential oil of lemon peels. Only six compounds of this group were identified and among them α-bergamotene and bisabolene were present at a concentration above 1%. These two sesquiterpenes were also identified in the volatile profile of essential oils obtained from five selected varieties of *C. limon* [30].

Similar to the lemon essential oil, oil obtained from clove buds is used as a food flavoring agent [31], in the preparations for gums and teeth, and in aromatherapy. It has the potential to be used as a natural preservative due to its antioxidant, antimicrobial, and antifungal activities [32,33]. As shown in Table 1, a total of 34 compounds, mainly belonging to the class of sesquiterpene hydrocarbons (10.71%) and others (83.91%), were identified in the clove essential oil. The major constituents of this oil were eugenol (62.74%), eugenol acetate (15.97%), caryophyllene (6.24%), humulene (3.54%), and chavicol (1.36%), while the content of the remaining components did not exceed 1%. Eugenol, eugenol acetate, caryophyllene, and humulene were also identified as the main components in the essential oil of cloves cultivated in the Mediterranean region of Turkey [34] and clove oil from Toli-Toli and Bali [35]. Our results were comparable to those reported for clove oil obtained from Toli-Toli, especially in terms of the content of eugenol and eugenol acetate, while the Turkish clove bud oil was richer in eugenol (87%) and contained less eugenol acetate compared to the content of these components determined in this study. In turn, the clove bud oil from Bangladesh was shown to consist of 31 compounds, of which eugenol was dominant. In comparison to our research, the percentage of eugenol was lower and amounted to 49.71% [36]. However, the content of this compound was about 15% higher in the oil obtained from clove leaves. Other studies showed that this bioactive compound was the most abundant at the flowering stage in mature trees [37]. On the other hand, the oil obtained from the stem of the clove plant in Amboina Island was characterized by a higher

percentage of eugenol compared to the oil obtained from the buds and leaves of the clove plant in the same region but a lower percentage of eugenol acetate and caryophyllene [38]. Amelia et al. [39] indicated that the chemical composition of the clove essential oil may be influenced by the geographic origin of plant population and the growing conditions. The authors revealed the differences between the content of major and minor constituents in Java and Manado clove oils. Clove oil from Java (55.60%) contained a lower amount of eugenol compared to Manado oil (74.64%) but a higher amount of eugenol acetate (20.54%). According to the results of our study, the content of these two compounds was between the lowest and the highest values reported for clove oils from Java and Manado. The presence of eugenol acetate may contribute more to the sweet aroma of clove oil, while eugenol and chavicol contribute to its spicy odor.

Table 1 presents the data on the yield of the three studied essential oils obtained by steam distillation. Clove oil was obtained with the highest yield (3.65%), while the yield of lemon peel oil was the lowest (0.63%). In turn, the yield of juniper berry oil was 0.95%. The chemical composition of the studied etheric oils and their yields could be influenced by seasonal variation, environmental conditions, the age of the plant, the latitude and altitude of growing site, the degree of maturity of the raw material used for distillation, and the part of the plant and method used to isolate the essential oil [37,39,40].

3.2. The Effect of Essential Oils on the Viability of Bifidobacterium in In Vitro Conditions

Each of the surveyed essential oils was tested for antibacterial action. Some of the cited studies have indicated that Gram-positive bacteria are more sensitive to these essential oils [7,11]. Thus, it could be assumed that the studied 7ifidobacterial strains, belonging to the Gram-positive bacterial group, should be inhibited by these oils. Table 2 presents the sizes of the inhibition zones observed for the studied bifidobacteria strains under the influence of the tested essential oils.

Two of the tested bifidobacteria strains (strains 45/03 and 89) were the most influenced by the essential oil of the lemon peel. Their growth inhibition zones differed from that of the remaining tested strains, as revealed by significant differences in the statistical analyses. Strain 89 showed the largest average inhibition zone, and the inhibition zone of strain 45/03 was only slightly smaller (Table 2) The average sizes of the zones of these strains were 0.98 and 0.96 mm, respectively. The other strains did not show a sensitivity to the lemon peel essential oil and their mean growth inhibition zones, observed with all the used concentrations of essential oils, were 0 mm. The obtained results indicate that the size of the bacterial growth zone is significantly determined by the bacterial strain. The maximum size of the zone of growth inhibition observed with this essential oil was, at the same time, the smallest when compared with the largest zones of growth inhibition observed with the other tested oils.

Table 1. Chemical compositions of essential oils from lemon peel (*C. limon* L. Burm.), clove bud (*S. aromaticum* L.), and juniper berry (*J. communis* L.) determined by GC–MS.

No.	Compound	KI [1]	Content (%) [2]			No.	Compound	KI	Content (%)		
			Lemon Peel	Clove Bud	Juniper Berry				Lemon Peel	Clove Bud	Juniper Berry
1.	2,3-Hexanedione	793	- [3]	-	0.42	61.	(E,E)-2,4-Decadienal	1314	-	-	0.22
2.	2-Heptanone	889	-	0.62	-	62.	Methyl geranate	1323	-	-	0.49
3.	Heptanal	896	0.05	-	-	63.	Chavicol acetate	1329	-	0.13	-
4.	α-Tricyclene	926	-	-	0.31	64.	Citronellol acetate	1350	0.25	-	
5.	α-Thujene	926	1.34	-	3.12	65.	α-Terpinyl acetate	1351	-	-	0.39
6.	α-Pinene	934	2.68	-	5.71	66.	α-Cubebene	1351	-	0.04	1.28
7.	Camphene	945	0.17	-	0.63	67.	Neryl acetate	1354	1.81	-	-
8.	Sabinene	969	3.06	-	6.19	68.	Eugenol	1356	-	62.74	-
9.	β-Pinene	972	7.14	-	3.59	69.	Geranyl acetate	1373	1.84	-	-
10.	β-Myrcene	985	4.49	-	6.45	70.	α-Copaene	1376	-	0.35	-
11.	Decane	996	-	0.08	-	71.	Tetradecene	1390	0.02	-	-
12.	Ethyl hexanoate	998	-	0.05	-	72.	Tetradecane	1398	0.08	-	-
13.	Octanal	999	0.66	-	-	73.	β-Elemene	1391	-	-	2.01
14.	α-Phellandrene	1002	0.28	-	0.67	74.	Dodecanal	1399	0.14	-	-
15.	δ-3-Carene	1007	0.02	-	0.73	75.	α-Gurjunene	1407	-	-	0.49
16.	α-Terpinene	1019	0.99	-	1.61	76.	Longifolene	1408			0.13
17.	p-Cymene	1026	4.06	-	3.98	77.	Caryophyllene	1408	0.45	6.24	1.54
18.	Eucalyptol	1031	-	0.02	-	78.	β-Copaene	1416	-	-	0.27
19.	Limonene	1033	31.51	0.04	4.78	79.	α-Bergamotene	1430	1.21	-	-
20.	2-Heptanol acetate	1044	-	0.71	-	80.	Aromadendrene	1439	-	-	0.13
21.	Ocimene	1047	0.26	0.11	-	81.	Cadina-3,5-diene	1437	-	-	0.14
22.	γ-Terpinene	1059	12.53	-	1.81	82.	α-Farnesene	1508	0.23	-	1.67

Table 1. *Cont.*

No.	Compound	KI [1]	Content (%) [2]			No.	Compound	KI	Content (%)		
			Lemon Peel	Clove Bud	Juniper Berry				Lemon Peel	Clove Bud	Juniper Berry
23.	1-Octanol	1062	0.05	-	-	83.	Humulene	1454	-	3.54	3.68
24.	cis-Sabinene hydrate	1069	0.08	-	-	84.	cis-Muurola-4(15),5-diene	1460	-	-	0.34
25.	cis-Linalool Oxide	1071	0.08	-	-	85.	Cadina-1(6),4-diene	1485	-	-	0.45
26.	Terpinolene	1085	1.06	-	-	86.	α-Muurolene	1499	-	0.13	1.26
27.	2-Nonanone	1091	-	0.45	-	87.	Germacrene D	1480	-	-	1.54
28.	Methyl benzoate	1091	-	0.24	-	88.	β-Cubebene	1420	-	-	2.88
29.	Linalool	1095	2.76	-	1.03	89.	Pentadecane	1450	0.14	0.13	-
30.	Nonanal	1110	0.73	-	-	90.	β-Selinene	1485	-	-	0.41
31.	Citronellal	1149	0.74			91.	Valencene	1489	0.19	-	-
32.	Acetic acid, phenylmethyl ester	1170	-	0.26		92.	Bicyclosesquiphellandrene	1482	-	-	1.61
33.	(Z)-p-Menth-2-en-1-ol	1188	-	-	0.76	93.	γ-Amorphene	1483	-	0.04	1.17
34.	α-Campholenal	1195	-	-	1.31	94.	α-Selinene	1494	-	0.26	-
35.	(E)-Pinocarveol	1139	-	-	0.75	95.	β-Elemene	1391	-	-	0.42
36.	Camphor	1143	-	-	0.59	96.	Bisabolene	1499	1.97	-	-
37.	α-Phellandren-8-ol	1166	-	-	2.78	97.	γ-Cadinene	1512	-	0.17	1.74
38.	Pinocarvone	1162	-	-	0.23	98.	δ-Cadinene	1510	0.04	-	3.50
39.	Terpinene-4-ol	1177	2.06	0.06	-	99.	Eugenol acetate	1524	-	15.97	-
40.	p-Cymen-8-ol	1180	-	-	2.34	100.	Epizonarene	1497	-	-	0.55
41.	α-Terpineol	1186	3.04	-	-	101.	Cadine-1,4-diene	1532	-	-	1.29
42.	Hydroxymethyl 2-hydroxy-2-methylpropionate	1189	-	-	2.29	102.	α-Amorphene	1506	-	-	0.56
43.	Methyl salicylate	1190	-	0.92	-	103.	α-Calacorene	1542	-	0.11	0.87

Table 1. *Cont.*

No.	Compound	KI [1]	Content (%) [2]			No.	Compound	KI	Content (%)		
			Lemon Peel	Clove Bud	Juniper Berry				Lemon Peel	Clove Bud	Juniper Berry
44.	Myrtenal	1193	-	-	0.67	104.	Germacrene B	1556	-	-	1.76
45.	Decanal	1203	0.13	-	-	105.	Spathulenol	1576	-	-	0.67
46.	Octyl acetate	1210	0.04	-	-	106.	Caryophyllene oxide	1581	-	0.96	0.68
47.	Verbenone	1214	-	-	1.42	107.	Humulene epoxide	1606	-	0.09	0.51
48.	Chrysanthenyl acetate	1232	-	-	0.12	108.	Di-epi-1,10-cubenol	1614	-	-	0.70
49.	Cumin aldehyde	1238	-	-	0.28	109.	α-Cadinol	1653	-	-	2.83
50.	Neral (E-Citral)	1239	2.74	-	-	110.	Cadalene	1674	-	-	0.39
51.	Carvone	1239	0.46	0.13	0.52	111.	Oplopanone	1733	-	-	1.02
52.	Chavicol	1253	-	1.36	-	112.	Benzyl benzoate	1762	-	0.34	-
53.	Geraniol	1253	3.38	-	-	113.	α-Copaene-11-ol	1891	-	-	0.15
54.	Methyl citronellate	1261	-	-	0.62	114.	Biformene	1979	-	-	0.16
55.	Geranial (Z-Citral)	1266	4.05	-	-	115.	m-Camphorene	1944	-	-	0.39
56.	Perilla aldehyde	1268	0.31	-	-	116.	p-Camphorene	1977	-	-	0.19
57.	Bornyl acetate	1280	0.07	-	0.78	117.	13-Epimanool	2056	-	-	0.65
58.	Thymol	1290	0.05	-	-	118.	Squalene	2790	-	0.08	-
59.	2-Undecanone	1291	-	0.09	0.85	119.	Heptacosane	2705		0.09	-
60.	Undecanal	1309	0.10	-	-	120.	Octacosane	2804		0.21	-
Monoterpene hydrocarbons (%)									68.53	0.26	39.58
Oxygenated monoterpenes (%)									24.78	0.66	15.08
Sesquiterpene hydrocarbons (%)									4.09	10.71	31.69
Oxygenated sesquiterpene (%)									-	1.05	6.56
Other (%)									2.14	83.91	5.56
Total identified (%)									99.54	96.59	98.47
Oil yield (%)									0.63	3.65	0.95

[1] Kovats' Retention Index on DB-5MS column; [2] relative content expressed as percentage of the total oil composition obtained by GC/MS; [3] not identified.

Table 2. Size of the growth inhibition zones (mm) of *Bifidobacterium* strains observed with selected essential oils (n = 4).

Bifidobacteria Strain	The Concentration of the Essential Oil [%]	Lemon Peel		Clove Bud		Juniper Berry	
		Mean	±SD (mm)	Mean	±SD (mm)	Mean	±SD (mm)
HN 019	100	0.0	±0.0 ^{a}	5.6	±0.4 ^{i,j}	0.0	±0.0 ^{a}
	75	0.0	±0.0 ^{a}	3.0	±0.2 ^{e,f}	0.0	±0.0 ^{a}
	50	0.0	±0.0 ^{a}	3.0	±0.1 ^{e,f}	0.0	±0.0 ^{a}
	25	0.0	±0.0 ^{a}	2.6	±0.7 ^{e}	0.0	±0.0 ^{a}
	0	0.0	±0.0 ^{a}	0.0	±0.0 ^{a}	0.0	±0.0 ^{a}
AD 50	100	0.0	±0.0 ^{a}	5.6	±0.5 ^{i,j}	1.8	±0.1 ^{c,d}
	75	0.0	±0.0 ^{a}	5.0	±1.2 ^{h,i}	0.0	±0.0 ^{a}
	50	0.0	±0.0 ^{a}	4.0	±0.0 ^{f,g}	0.8	±0.9 ^{b}
	25	0.0	±0.0 ^{a}	4.5	±1.4 ^{g}	0.6	±0.8 ^{a,b}
	0	0.0	±0.0 ^{a}	0.0	±0.0 ^{a}	0.0	±0.0 ^{a}
45/03	100	1.8	±1.0 ^{c,d}	4.0	±0.5 ^{f,g}	1.9	±0.7 ^{c,d}
	75	1.5	±1.0 ^{c,d}	2.7	±0.4 ^{e}	1.3	±1.0 ^{c}
	50	1.0	±1.0 ^{b}	3.4	±0.3 ^{e,f}	1.0	±1.0 ^{b,c}
	25	0.5	±0.6 ^{a,b}	1.1	±0.5 ^{b,c}	0.4	±0.5 ^{a,b}
	0	0.0	±0.0 ^{a}	0.0	±0.0 ^{a}	0.0	±0.0 ^{a}
AD 600	100	0.0	±0.0 ^{a}	7. 8	±2.6 ^{k}	3.3	±1.8 ^{e,f}
	75	0.0	±0.0 ^{a}	7.7	±2.3 ^{k}	2.8	±1.3 ^{e,f}
	50	0.0	±0.0 ^{a}	3.5	±0.3 ^{e,f}	0.8	±0.9 ^{b}
	25	0.0	±0.0 ^{a}	1.2	±1.0 ^{b,c}	0.0	±0.0 ^{a}
	0	0.0	±0.0 ^{a}	0.0	±0.0 ^{a}	0.0	±0.0 ^{a}
KNA 1/08	100	0.0	±0.0 ^{a}	10.4	±4.2 ^{m}	3.5	±1.2 ^{e,f}
	75	0.0	±0.0 ^{a}	8.6	±4.8 ^{l}	3.3	±0.7 ^{e,f}
	50	0.0	±0.0 ^{a}	5.5	±0.1 ^{i}	2.8	±0.7 ^{e,f}
	25	0.0	±0.0 ^{a}	4.8	±0.5 ^{g,h}	2.2	±1.1 ^{d,e}
	0	0.0	±0.0 ^{a}	0.0	±0.0 ^{a}	0.0	±0.0 ^{a}
Bb-12	100	0.0	±0.0 ^{a}	14.9	±2.1 ^{n}	4.6	±2.2 ^{g,h}
	75	0.0	±0.0 ^{a}	14.5	±3.0 ^{n}	5.6	±0.3 ^{i}
	50	0.0	±0.0 ^{a}	0.0	±0.0 ^{a}	5.1	±1.3 ^{h,i}
	25	0.0	±0.0 ^{a}	10.7	±2.9 ^{m}	4.9	±0.7 ^{h}
	0	0.0	±0.0 ^{a}	0.0	±0.0 ^{a}	0.0	±0.0 ^{a}
89	100	1.8	±1.1 ^{c,d}	5.8	±0.0 ^{j}	2.9	±1.0 ^{e,f}
	75	1.3	±1.0 ^{c}	3.6	±0.3 ^{e,f}	3.2	±1.1 ^{e,f}
	50	1.3	±1.0 ^{c}	2.0	±0.0 ^{d}	2.3	±1.0 ^{e}
	25	0.5	±0.6 ^{a,b}	4.5	±1.3 ^{g,h}	1.4	±1.0 ^{c}
	0	0.0	±0.0 ^{a}	0.6	±0.3 ^{a,b}	0.0	±0.0 ^{a}

Means with different lowercase letters (a–n) are significantly different ($p < 0.05$) using a three-way ANOVA.

Fisher and Philips [41] showed that the essential oils obtained from sweet oranges and lemons exhibited effective antibacterial activities compared to bergamot oil, citral, and linalool. The lemon essential oil contained the largest amount of limonene, but the lowest amount of citral compared to the essential oils obtained from sweet oranges and bergamot. Limonene did not show antibacterial action. The authors also stated that the Gram-positive bacteria were more sensitive to the antibacterial action of essential oils compared to the Gram-negative bacteria. Among the studied Gram-positive bacteria, *S. aureus* was found to be the most resistant. The results of previous research [41] confirmed that of our work, which showed that the lemon oil was characterized by weak antibacterial action. Prabuseenivasan et al. [42] tested the effect of 21 different essential oils on four species of Gram-negative bacteria (*E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Proteus vulgaris*) and two species of Gram-positive bacteria (*Bacillus subtilis* and *S. aureus*) and showed that lemon oil moderately inhibited the growth of the tested bacteria.

Bevilacqua et al. [43] referred to the research of other authors on the influence of essential oils on the growth of microorganisms. Based on the studies that analyzed the antibacterial effects of essential oils and their active substances, these authors argued that the bioactivity of essential oils was generally associated with phenolic compounds. Phenolic compounds dissolve in a lipid diaphragm layer and change their membrane liquidity, as well as causing the leakage of intracellular components and the scattering of transmembrane gradient H^+. Thus, the inhibitory effect of the citrus extract on the growth of bacteria can be linked with the substances it contains. Citrus extract was found to be a natural source of antioxidants, flavonoids (rutin, naringin, quercetin, and naringenin), and other phenolic compounds, such as limonene, linalool, and citral. Therefore, it has been proposed that the bioactivity of the citrus extract may be the effect of the synergistic activity of these components. The results reported by Prabuseenivasana et al. [42] on of the antibacterial effects of lemon essential oils differed from that obtained in our work. Prabuseenivasan et al. [42] showed that lemon oil exhibited a stronger antibacterial effect than that observed in the present work. This could be due to the differences in the methods used for analyses, as well as the bacterial species tested. In their study on the influence of lemon essential oils on anaerobic bacteria that affect the teeth and oral cavity, which were grown from materials collected from patients, Kędzia et al. [44] found that the essential oil decreased the number of the analyzed bacteria. These results confirmed that Gram-positive bacteria were more sensitive to the antibacterial effects of lemon essential oils compared to Gram-negative bacteria. Among the Gram-positive bacteria group analyzed by Kędzia et al. [44], there were two *B. breve* strains, and these bacteria were inhibited by the citric essential oil. The minimum inhibitory concentration (MIC) values determined for both strains were the same, and amounted to 15 mg/mL. In summary, lemon oil inhibited the growth of certain types of bacteria, with a stronger effect on Gram-positive bacteria. However, its activity was weaker compared to the essential oils obtained from other plants such as cloves, cinnamon, and bergamot.

In our study, the clove bud essential oil was particularly distinguished from other tested essential oils due to the size of the bacterial growth zone (Table 2). This essential oil had a statistically significantly higher inhibitory effect on the growth of all tested *Bifidobacterium* strains. Compared to the other two essential oils, clove oil appeared to have significantly higher amounts of carnation oil.

Bevilacqua et al. [43] showed that eugenol did not inhibit the growth of *Lactobacillus* bacteria (*L. plantarum* and *L. brevis*). Badei et al. [45] compared the antimicrobial activity of three spices, cardamom, cinnamon, and clove, and essential oils obtained from them, using 13 strains of Gram-positive and Gram-negative bacteria, seven fungal strains, and two yeast strains. They showed that the clove essential oil had the strongest effect. Clove oil also showed stronger antimicrobial activity than phenol. Furthermore, this essential oil was one of the three tested essential oils that showed antibacterial effects on pathogenic *S. aureus* bacteria. These results confirm the strong antibacterial effect of clove bud essential oils observed in our work. It can be assumed that the antibacterial effect of clove bud essential oils may be related to the content of the active substances present in it. The most active and abundant component in the clove essential oil is eugenol, which has proven antibacterial, as well as antifungal, effects. Makuch et al. [46] also confirmed the antimicrobial action of the clove essential oil on the yeast-like fungi *Candida albicans*, the Gram-negative bacteria *E. coli*, and the Gram-positive bacteria *Staphylococcus epidermidis*, as well as the high content of eugenol in this oil. The average size of the growth inhibition zones observed in their study on *C. albicans*, *E. coli*, and *S. epidermidis* was 11.76 $\pm$ 0.7, 5.16 $\pm$ 0.2, and 5.10 $\pm$ 0.3 mm, respectively. The results presented by Makuch et al. [46] are in line with that of our work on the activity of the clove bud essential oil relative to Gram-positive bacteria. Liu et al. [47] performed many studies confirming the strong antimicrobial activity of clove essential oils. Ayoola et al. [48] and Ali et al. [49] also studied the composition and antimicrobial activity of clove essential oils. Similar to our study, the results of Ayoola et al. [48] showed that eugenol is the main component of the clove essential oil, and caryophyllene, eugenol acetate, and

α-humulene are also abundant. The cited researchers also showed the inhibitory effects of oil on Gram-positive and Gram-negative bacteria, as well as yeast-like fungi, with the smallest growth inhibition zone observed for *Enterobacter cloacae* and *E. coli* bacteria (10 mm) and the largest for the yeast-like fungi *C. albicans* (35 mm). Gram-positive bacteria (*S. aureus*) were shown to be more sensitive to clove essential oils (the largest growth inhibition zone was 23 mm), where the largest inhibition zones were recorded for samples treated with the largest concentration of the clove essential oil.

Based on the strength of the inhibitory effects on the growth of *Bifidobacterium* strains studied in in vitro conditions, juniper berry essential oil ranked second among the three tested essential oils. The highest size of the bifidobacterial growth inhibition zone observed with this essential oil was 5.6 mm (Table 2). Statistical analyses revealed that the size of growth inhibition zones depends on the *Bifidobacterium* bacterial strain tested. The most sensitive strain was *B. animalis* subsp. *lactis* Bb-12. With the addition of 25% juniper berry essential oil, the size of the growth inhibition zone of the strain observed was 4.9 mm and it increased with the increase in the concentration of the essential oil. At a concentration of 50% and 75%, the average growth inhibition zone was 5.1 and 5.6 mm, respectively. A slight reduction in the growth inhibition zone was noted at a concentration of 100% and the size of the zone was 4.6 mm. However, HN 019 strains did not show sensitivity to the antibacterial effects of the juniper berry essential oil, because the average growth inhibition zones observed with the use of each concentration was 0 mm.

Şerban et al. [50] used two species of bacteria (*E. coli* and *S. aureus*) and one species of yeast (*C. albicans*) to determine the properties of the juniper berry essential oil and several other essential oils. The authors recorded growth inhibition zones of 14, 12, and 17 mm for *E. coli, S. aureus*, and *C. albicans*, respectively, using juniper berry essential oils. They observed larger growth inhibition zones of the analyzed microorganisms with the use of the juniper berry essential oil, compared to our work. This may be due to differences in the methods used for assessing growth inhibition, the sensitivity of microorganisms, and the composition of individual essential oils. Rezvani et al. [51] analyzed the composition of the juniper berry essential oil and the antibacterial activity of this oil by the well diffusion method using *S. aureus, P. Aeruginosa*, and *E. coli*. They identified 30 compounds but the GC–MS analysis showed that the main component of the juniper berry essential oil was α-piren (45.63%), which is in line with the research of other authors [52]. The results reported by Pepeljnjak et al. [10] also confirmed the results obtained in our work. In the study by Rezvani et al. [51], the juniper berry essential oil showed an antibacterial effect on all three tested bacteria, with *P. aeruginosa* found to be the most resistant. A growth inhibition zone of 4.5 mm was observed only with the use of a 100% concentration of essential oil. The most sensitive among the tested bacteria was *E. coli* and its growth inhibition zones that were observed for concentrations of essential oils of 0%, 10%, 20%, 50%, and 100% were measured at 0, 6.1, 7.2, 8.3, and 2.2 mm, respectively. The inhibition zones of the Gram-positive *S. aureus*, similar to the *Bifidobacterium* strains tested in this work, that were observed with 0%, 10%, 20%, 50%, and 100% concentrations of essential oils were measured at 0, 2.1, 4.8, 5.0, and 3.5 mm, respectively. These results on *S. aureus* are very similar to those found in our work for *B. animalis* subsp. *lactis* Bb-12, which turned out to be the most sensitive.

Some researchers have shown that essential oils obtained from juniper berries did not show any antibacterial action and only exhibited an antifungal effect [11], or weak antibacterial and antifungal activities [53]. Glišić et al. [54] examined the antibacterial activity of the juniper berry essential oil, with different fractions, in their study. These authors reported that the native juniper berry essential oil did not show a strong antibacterial effect compared to fractions containing a high amount of α-pinene and a mixture of sabinene and α-pinene, which showed the highest antimicrobial activity on almost all tested microorganisms (*B. cereus, E. coli, Listeria monocytogenes, Corynebacterium* sp., *P. aeruginosa, S. aureus, C. albicans, Alternaria* sp., *Aspergillus nidulans*, and *Aspergillus niger*). The MIC values estimated by Prabuseenivasana et al. [42] for 21 different essential oils and

four species of Gram-negative bacteria (*E. coli, K. pneumoniae, P. aeruginosa*, and *P. vulgaris*) and two species of Gram-positive bacteria (*B. subtilis* and *S. aureus*) indicated that these results are similar to those obtained in our work. For Gram-positive bacteria, the MIC value of the lemon essential oil (12.8 and 12.8 mg/mL, respectively) was higher than that of the clove essential oil (6.4 and 3.2 mg/mL, respectively) which confirms that the clove essential oil has a stronger antibacterial effect than the lemon essential oil. The results obtained in our work, to some extent, coincide with those of Bevilacqua et al. [43], who studied the antimicrobial activity of a eugenol extract (the main component of the clove essential oil, limonene),the main component of the lemon essential oil, and a citrus extract (a mixture of different components such as citric acid, sodium acid, citrusic polyphenols, bioflavoproteins, citrus sugars, and citric acid) on three bacterial species, including two lactic acid bacteria (LAB), and three yeast species. The MIC values determined by Bevilacqua et al. [43] indicated that limonene showed less antimicrobial activity than eugenol on the tested bacteria (>1000 and >600 ppm, respectively). It should be noted that several compounds that were identified as strong antioxidants belong to the group of flavonoids found in the citrus extract, as well as the groups of other phenolic compounds. Bevilacqua et al. [43] showed that these substances acted synergistically. It can be argued that limonene, as a single compound, did not exhibit as strong a antimicrobial effect as the citrus extract that contained many additional compounds that synergistically increased its antimicrobial effect. This suggests that the action of a particular essential oil on a given bacterial species depends on the composition of the essential oil, the interaction between its components, and the concentration of the essential oil used. The effect of an essential oil is also influenced by the conditions under which its activity is tested and by the bacterial species and strains used for the analysis, as each strain is different and exhibits different sensitivities. Hammer et al. [55] emphasized that it is difficult to compare the results obtained by various authors regarding the antibacterial action of different essential oils. This is due to the fact that the composition of oils obtained from the same plants may differ due to changes that occur in plants under the influence of climatic and environmental factors. Essential oils with the same name can often be produced from different plant species. Moreover, the type of method used to measure the sensitivity of microorganisms to the essential oil is of great importance. Depending on the methods used, the tested microorganisms may experience different growth conditions and may be exposed to the essential oil to different degrees. The solubility of the emulsifier and essential oil, as well as its components in a given environment, may also vary. To the best of our knowledge, studies such as those performed in our work, i.e., using a well-diffusion method and exploring the influence of various concentrations of clove bud, lemon peel, and juniper berry essential oils on the survival of *Bifidobacterium* bacteria have not been conducted earlier (the well-diffusion method is applied in experiments concerning plant extracts rather than essential oils).

3.3. The Effect of Essential Oil Concentrations on the Viability of B. animalis Subsp. lactis Bb-12 in Cream during Fermentation and after 21 Days of Storage

The strain *B. animalis* subsp. *lactis* Bb-12 that was selected for the experiments was identified as the most sensitive to the tested essential oils. If there was no influence of the tested essential oils and their concentrations on the viability of the most sensitive strain (*B. animalis* subsp. *Lactis* Bb-12) in the cream, we assumed, with a high degree of certainty, that the other tested bifidobacterial strains would also not show a sensitivity to the tested essential oils added to the fermented cream. The number of *B. animalis* subsp. *lactis* Bb-12 cells present in our samples of fresh cream (before fermentation) ranged from 8.1 to 8.3 log(CFU/mL), regardless of the type of oil used and the concentration of the oil tested (Table 3). After fermentation, a change in the population of bifidobacterial cells was recorded and the statistical analyses showed that the final population of *B. animalis* subsp. *Lactis* Bb-12 cells in the obtained sour cream was not influenced by the type of essential oils, or the concentration used. The sour cream samples were stored for 21 days under refrigeration conditions to investigate the deferred or cumulative influence of the tested

essential oils on the viability of *B. animalis* subsp. *lactis* Bb-12. It was noted that refrigerated storage caused a statistically significant reduction in the population of *B. animalis* subsp. *lactis* in the sour cream samples; however, the dynamics of changes did not depend on the type of essential oils, or the concentration of the oils used. In all stored sour cream samples, the number of live *B. animalis* subsp. *lactis* Bb-12 cells ranged from 7.5 to 7.8 log(CFU/mL).

Table 3. Effects of selected essential oils on *B. animalis* subsp. *lactis* Bb-12 population (expressed as log(CFU/mL)) in cream and sour cream samples (n = 4).

The Concentration of the Essential Oil Added to Cream	Samples	Lemon Peel		Clove Bud		Juniper Berry	
		[Log(CFU/mL)]		[Log(CFU/mL)]		[Log(CFU/mL)]	
		Mean	±SD	Mean	±SD	Mean	±SD
100%	Cream	8.3	±0.4 ^{a}	8.2	±0.4 ^{a}	8.3	±0.4 ^{a}
	Sour cream	9.3	±0.1 ^{b}	9.3	±0.1 ^{b}	9.1	±0.1 ^{b}
	Stored sour cream	7.8	±0.1 ^{c}	7.6	±0.1 ^{c}	7.5	±0.1 ^{c}
50%	Cream	8.3	±0.4 ^{a}	8.3	±0.4 ^{a}	8.1	±0.4 ^{a}
	Sour cream	9.3	±0.1 ^{b}	9.3	±0.1 ^{b}	9.2	±0.0 ^{b}
	Stored sour cream	7.7	±0.2 ^{c}	7.7	±0.1 ^{c}	7.6	±0.2 ^{c}
25%	Cream	8.2	±0.4 ^{a}	8.3	±0.4 ^{a}	8.2	±0.4 ^{a}
	Sour cream	9.5	±0.1 ^{b}	9.3	±0.1 ^{b}	9.4	±0.0 ^{b}
	Stored sour cream	7.6	±0.4 ^{c}	7.7	±0.2 ^{c}	7.6	±0.3 ^{c}
12.5%	Cream	8.3	±0.4 ^{a}	8.2	±0.4 ^{a}	8.3	±0.4 ^{a}
	Sour cream	9.4	±0.2 ^{b}	9.4	±0.0 ^{b}	9.3	±0.0 ^{b}
	Stored sour cream	7.7	±0.2 ^{c}	7.5	±0.3 ^{c}	7.7	±0.1 ^{c}
0%	Cream	8.1	±0.4 ^{a}	8.3	±0.4 ^{a}	8.2	±0.4 ^{a}
	Sour cream	9.3	±0.2 ^{b}	9.3	±0.2 ^{b}	9.3	±0.2 ^{b}
	Stored sour cream	7.8	±0.1 ^{c}	7.7	±0.1 ^{c}	7.8	±0.1 ^{c}

Means with different lowercase letters (a–c) are significantly different ($p < 0.05$) using a three-way ANOVA.

Our previous study examined the effect of commercial essential oils on the growth of selected LAB [56,57], as well as the effect of plant extracts on the populations of LAB, which showed that LAB cultures tolerated the extracts present in milk well, at an amount of up to 3.0% [58]. The effects of coriander essential oils (concentrations from 1% to 100%) on the growth inhibition of the studied *Lactobacillus* bacteria (7 *L. acidophilus* strains, 7 *L. casei* strains, 3 *L. delbrueckii* strains, 3 *L. plantarum* strains, 3 *L. rhamnosus* strains, *L. fermentum*, *L. helveticus,* and *L. paracasei*) were tested using the well diffusion method. The growth inhibition zones did not exceed 6.3 mm. The low sensitivity of LAB to coriander oil favored the production of fermented products by these bacteria [58]. Until now, the influence of essential oils or plant extracts on the viability of bifidobacteria has not been investigated. The available data in the literature mainly related to LAB and other essential oils, not those studied in our work, and only a few studies included bifidobacteria in this subject of research.

Shahdadi et al. [59] determined the survival of *L. acidophilus* LA-5 and *B. animalis* subsp. *lactis* Bb-12 in stored probiotic yogurt drinks enriched with essential oils from green mint (*Mentha spicata*), eucalyptus (*Eucalyptus camaldulensis*), long-leaf mint (*Mentha longifolia*), and *Ziziphora tenuior* L. The authors found that the number of bacterial cells decreased during the storage of yogurts, and yogurts containing the eucalyptus essential oil showed the highest inhibitory effect. It is worth mentioning that the largest number of *L. acidophilus* LA-14 and *B. animalis* subsp. *lactis* Bb-12 cells were observed in yogurts containing the green mint essential oil. Interestingly, *B. animalis* subsp. *lactis* showed better survival rates in all yogurt samples compared to *L. acidophilus*. The results presented by Shahdadi et al. [59] confirmed those obtained in our work despite the differences, among others, in the types of essential oils and dairy products tested. Voosogh et al. [60] also found a decrease in the number of *B. lactis* Bb-12 and *L. acidophilus* LA-5 during storage

in an Iranian milk drink (doogh) containing essential oils from mint. The essential oil was used at two concentrations (1% and 2%) and its effect on the number of bacterial cells was found to depend on its concentration. Similar to Shahdadi et al. [59], Voosogh et al. [60] noticed that *L. acidophilus* were more sensitive than *B. lactis*. After 8 weeks of storage, the number of bifidobacterial cells in the milk drink decreased by 2 log(CFU/mL), while the number of lactobacilli cells was below the detection limit. This confirms the higher viability of *B. animalis* subsp. *lactis* Bb-12, as observed in our work. Panesar and Shinde [61] also studied the viability of probiotic *L. acidophilus* and *B. bifidum* bacteria, along with cultures of yogurt bacteria in the yogurt-containing *Aloe vera* extract, and found that the *Bifidobacterium* population was higher than the lactobacilli population. Bayoumi [62] analyzed the influence of four essential oils, clove, cardamom, cinnamon, and peppermint, on the growth of various LAB in yogurts. They found that the tested bacteria were quite sensitive to all the studied essential oils and that the sensitivity of the tested microorganisms depended on the concentration of oil and the bacteria used.

Smith-Palmer et al. [63] proposed several theories explaining the influence of essential oils on microorganisms in various food products. One of the theories was based on fat content. Fat has the ability to form a protective layer around the bacterial cells in food and protects them against the action of essential oils. According to another theory, fat can absorb the essential oil used and can reduce its content in the aqueous fraction and, thus, its antimicrobial effect. This can explain the difference between our observations and the results of Khodaparast et al. [64] and Behard et al. [65] who used yogurt in studies, while our work was based on cream. The fat content in yogurt varies from 0.0% to 4.5%, while the cream used in the present work contained 36% fat. The number of probiotic bacteria cells found by Behard et al. [65] is smaller than the number of bacterial cells observed in sour cream samples in our work. Behard et al. [65] added cinnamon extract and liquorice to yogurt containing *L. acidophilus* LA-5 and found that the extract caused a reduction in the number of probiotic bacterial cells.

3.4. Fatty Acid Composition

GC–MS was used as a reliable method for the analysis of the fatty acid composition and the content of polyunsaturated, monounsaturated, and saturated fatty acids in the fat extracted from the cream samples fermented with *B. animalis* subsp. *lactis* Bb-12 and enriched with essential oils from the lemon peel, clove bud, and juniper berry. The fatty acid profile of the studied fat samples was determined after 21 days of storage at 6 °C and is presented in Table S1. The GC–MS analysis revealed the presence of 20 fatty acids. Saturated fatty acids were the dominant group in all the tested fat samples. In our work, their content ranged from 67.36% (fat extracted from cream not subjected to fermentation) to 68.32% (stored sour cream without essential oils). The most abundant saturated fatty acids were myristic acid (C14:0), palmitic acid (C16:0), and stearic acid (C18:0). The content of myristic acid (C14:0) was the greatest in fermented cream, containing 50% clove bud essential oils amounting to 11.84%, while in the remaining fat samples, it ranged between 11.58% and 11.78%. No statistically significant differences were noted in the content of this acid between the sour cream and stored sour cream samples. Moreover, the content of palmitic acid was slightly higher in samples fermented with *Bifidobacterium* compared to the nonfermented cream samples, except in the case of those samples enriched with lemon peel, clove bud, or juniper berry essential oils at their highest concentrations (100%). Similar observations were made regarding the content of stearic acid (C18:0) in the analyzed fat samples. Short-chain fatty acids (C4:0–C10:0) accounted for 6% of saturated fatty acids. Along with butyric (C4:0), caproic (C6:0), and caprylic acid (C8:0), capric acid (C10:0) was also found and its content was comparable in the analyzed fat samples, with the greatest amount noted in the fat extracted from sour cream samples enriched with 100% lemon peel and juniper berry essential oils and with 50% and 100% clove bud essential oils. The presence of C6:0, C8:0, and C10:0 fatty acids in ruminant milk may cause a specific aroma

in milk and may exert beneficial effects on human health by inhibiting bacterial and viral growth, as well as by dissolving cholesterol deposits [66].

Compared to saturated fatty acids, the content of monounsaturated fatty acids in the analyzed samples ranged from 28.24% to 29.07%. Among these acids, oleic acid (C18:1 *n*-9) was the most abundant and its share in the total composition of fatty acids was, on average, 21.85%. In the fat samples extracted from sour cream enriched with three essential oils at the level of 100%, compared to the fat samples from sour cream not containing essential oils, a slightly lower oleic acid content was found in the cream sample not treated with *B. animalis* subsp. *lactis* Bb-12 and in the sample enriched with essential oils, both after storage for 21 days and immediately after fermentation. The highest content of oleic acid was noted in the sour cream samples containing juniper berry and clove bud essential oils that were added to the fermented cream at concentrations of 12.5% and 25%, and the oleic acid content in these samples exceeded 22%. The other monounsaturated acids found were myristoleic (C14:1), palmitoleic (C16:1), gadoleic (C20:1), C18:1 11c, and C18:1 12c acids, in minor amounts.

Similarly, the content of polyunsaturated fatty acids in the fat separated from sour cream samples accounted for 3.22–3.58% of the total fatty acid composition. The highest total content of polyunsaturated fatty acids was found in the fat separated from nonfermented cream (3.58%) and the lowest was found in the sour cream samples enriched with juniper berry essential oils that were stored for 21 days. Among the polyunsaturated fatty acids, linoleic acid (C18:2 *n*-6) was dominant in all tested fat samples, with an average amount of 1.59%. The lowest content of this fatty acid was found in the sour cream samples enriched with the juniper berry essential oil, except for the samples enriched with the highest concentration of this essential oil (100%). In all the studied fat samples, conjugated linoleic acid C18:2 9c11t (CLA) was also found. Due to its unique structure, it inhibits the enzymes involved in the deposition of adipose tissue. It also reduces the synthesis of adipose tissue, intensifies lipolysis, and has been proven to exhibit anticancer, antidiabetic, anti-inflammatory, and anti-atherosclerotic properties [66,67]. In our study, the content of CLA in the extracted fat samples varied within narrow limits (0.85–0.93%), regardless of the type of fat studied. Both CLA and vaccenic acid (C18:1 11t), which is a precursor of CLA in human organisms, are the main trans fatty acids present in ruminant milk. Their content slightly exceeded 3% in the sour cream samples. The study conducted by Izsó et al. [68] showed that trans fatty acids accounted for slightly more than 2% of the fatty acid content of the sour cream samples.

Comparing the fatty acid profile of the cream fermented by the probiotic *Bifidobacterium* bacteria to that of nonfermented cream, one can notice the variations in the content of four monounsaturated fatty acids, as well as three fatty acids that differ in the degree of unsaturation. The cream fermented with *Bifidobacterium* was characterized by a higher content of myristic, palmitic, and stearic acid compared to nonfermented cream. However, it contained a lower amount of lauric, oleic, linoleic, and α-linolenic acid. The content of these acids also did not change significantly during their 21-day storage. In turn, Yilmaz-Ersan et al. [69] observed that during storage, cream fermented with *B. lactis* contained a higher amount of linoleic and α-linolenic acid compared to the control sample and the cream fermented with *B. lactis* had increased amounts of mono- and polyunsaturated fatty acids compared to the cream fermented with *L. acidophilus.* In turn, Laučienė et al. [70] observed that the profile of individual fatty acids did not change during the processing of sour cream samples, or their storage at 5 °C.

3.5. The Volatile Compound Profile of Cream and Sour Cream Samples

Some fermented foods are a rich source of bioactive components which may have beneficial effects on health. LAB used as starter cultures may allow the transformation of lactic acid and citrate, lactate, protein, and fats into volatile compounds which, along with amino acids and other products, may play a critical role in the flavor of the resulting products [71]. In our work, volatile compounds in cream samples enriched with essential

oils from the lemon peel, clove bud, and juniper berry that was fermented with *B. animalis* subsp. *lactis* Bb-12 were identified by SPME-GC–TOF-MS and the data are presented in Table 4. The results are presented as the average relative peak area of three replicates (the peak area of the compound divided by the peak area of internal standard naphthalene d8). These did not correspond with the absolute amount of a compound identified in the studied samples, but were calculated and used only for evaluating the differences between the analyzed samples. The use of the SPME-GC–TOF-MS method allowed us to identify 22 volatile compounds belonging to the classes of aldehydes, ketones, acids, sulfur compounds, terpenes, and phenolic constituents. Two sulfur compounds, five ketones, and one aldehyde were found in the cream samples not treated with LAB. The ketones were represented by the following compounds: 2-butanone, 2-pentanone, 2-hexanone, 2-heptanone, and 2-nonanone. They were identified as the most important aroma components of cheeses with mold growth [72]. Among them, 2-pentanone, 2-heptanone, and 2-nonanone are characterized by a fruity floral fragrance, while 2-butanone and 2-hexanone have a sharp sweet smell. This class of carbonyl compounds is formed by the enzymatic oxidation of free fatty acids to β-keto acids and their decarboxylation, although 2-butanone is formed from diacetyl (2,3-butanedione), which is a product of lactose fermentation and citrate transformation. Only the content of 2-heptanone and 2-nonanone was found to be changed in the cream samples fermented with *B. animalis* subsp. *lactis* Bb-12, while the level of the remaining ketones was comparable to the nonfermented cream samples. In general, except for the stored sour cream samples containing the highest concentration of clove bud essential oils, all stored sour cream samples showed no increase in the identified ketones compared to the samples at the initial day. Moreover, 2,3-butanedione (diacetyl), a diketone responsible for the buttery flavor of the dairy product, was present in a lower amount in stored sour cream samples compared to unstored sour cream samples. However, the sour cream samples enriched with 12.5% essential oils contained a similar amount of 2,3-butanedione as that of unstored sour cream. It should be noted that diacetyl is produced by some species of the LAB family, including *Streptococcus*, *Leuconostoc*, *Lactobacillus*, and *Pediococcus*. Lew et al. [73] found higher diacetyl production in lactobacilli compared to bifidobacteria. The strain *L. casei* BT 1268 contained higher amounts of diacetyl in both intracellular and extracellular extracts, while *B. animalis* subsp. *lactis* BB 12 and *B. longum* BL 8643 showed a lower concentration in both fractions. In addition, the content of diacetyl in a product may increase or decrease during fermentation. Such changes can be explained by the fact that diacetyl is highly volatile and can evaporate at low temperatures, as well as from dry products. Among the volatile compounds identified in all tested samples, there were two sulfur compounds, namely, hexanal (aldehyde) and dimethyl sulfide. Dimethyl sulfide determines the flavor of cheese and may be present in yogurts [74] in varying amounts depending on the technique used for yoghurt-making and whether skim milk or full-fat milk is used for yogurt production. Sulfur compounds can be formed from the degradation of methionine by enzymes released by LAB. In our study, the stored sour cream samples containing the essential oils of clove buds and juniper berries had a higher amount of hexanal than the stored sour cream samples without additives. On the other hand, essential oil-enriched sour cream samples that were fermented and stored for 21 days had an increased content of dimethyl sulfide.

Table 4. Volatile compounds identified in cream samples using SPME-GC–TOF-MS system.

Compound	IK [1]	Ion [2]	Cream	Sour Cream	Storage Sour Cream	Lemon Peel				Clove Bud				Juniper Berry			
						12.5%	25%	50%	100%	12.5%	25%	50%	100%	12.5%	25%	50%	100%
Acetaldehyde	438	43	- [3]	-	-	32 ± 1 [a,b]	32 ± 1 [a,b]	33 ± 1 [a,b]	29 ± 0 [a,b]	247 ± 23 [c]	34 ± 2 [a,b]	33 ± 2 [a,b]	28 ± 0 [a]	24 ± 2 [a]	35 ± 1 [b]	29 ± 1 [a,b]	27 ± 1 [a]
Methanethiol	446	47	31 ± 2 [c]	90 ± 7 [g]	52 ± 4 [f]	35 ± 2 [c]	34 ± 4 [c]	35 ± 2 [c]	28 ± 0 [b]	37 ± 1 [c,d]	42 ± 2 [e]	35 ± 2 [c]	25 ± 1 [b]	16 ± 1 [a]	38 ± 2 [d,e]	16 ± 3 [a]	16 ± 2 [a]
Dimethyl sulfide	525	47	130 ± 13 [e]	113 ± 9 [d,e]	68 ± 4 [a]	123 ± 2 [e]	117 ± 2 [d,e]	96 ± 3 [c]	78 ± 4 [b]	104 ± 2 [c,d]	124 ± 13 [e]	121 ± 7 [e]	80 ± 2 [b]	111 ± 4 [d]	112 ± 1 [d]	84 ± 8 [b]	56 ± 4 [a]
2-Butanone	579	72	376 ± 39 [f]	372 ± 49 [f]	251 ± 19 [e]	165 ± 4 [c,d]	183 ± 7 [d]	181 ± 4 [d]	134 ± 6 [b]	273 ± 1 [e]	223 ± 10 [e]	144 ± 16 [b,c]	189 ± 14 [d]	138 ± 9 [b]	179 ± 4 [d]	142 ± 3 [b]	94 ± 1 [a]
2,3-Butanedione	601	86	-	496 ± 35	387 ± 14	461 ± 5 [d]	263 ± 18 [b]	337 ± 13 [c]	170 ± 10 [a]	487 ± 23 [d]	422 ± 26 [d]	341 ± 31 [c]	165 ± 11 [a]	474 ± 41 [d]	330 ± 9 [c]	258 ± 10 [b]	144 ± 5 [a]
Acetic acid	638	60	-	931 ± 14 [a]	1542 ± 19 [c]	16,808 ± 1633 [c]	9939 ± 1001 [a,b]	7110 ± 5272 [a]	8360 ± 1635 [a,b]	13,098 ± 878 [b,c]	6162 ± 691 [a]	6159 ± 432 [a]	5761 ± 380 [a]	16717 ± 511	10,091 ± 7724 [a,b]	16,394 ± 540 [c]	13,825 ± 281 [b,c]
2-Pentanone	689	58	446 ± 23 [c]	414 ± 39 [b,c]	339 ± 10 [c]	318 ± 80 [a]	369 ± 3 [b]	302 ± 6 [a]	284 ± 7 [a]	345 ± 10 [a]	607 ± 17 [e]	499 ± 134 [c]	582 ± 73 [d]	341 ± 17 [a,b]	348 ± 4 [a,b]	280 ± 5 [a]	279 ± 3 [a]
Dimethyl disulfide	718	94	-	93 ± 8 [d]	23 ± 1 [c]	23 ± 2 [c]	10 ± 0 [a]	12 ± 0 [a,b]	11 ± 0 [a]	23 ± 1 [c]	25 ± 7 [c]	25 ± 2 [c]	14 ± 1 [b]	22 ± 1 [c]	11 ± 0 [a]	8 ± 0 [a]	12 ± 0 [a,b]
Hexanal	780	56	166 ± 24 [d]	414 ± 35 [f]	132 ± 7 [b]	142 ± 8 [b,c]	129 ± 7 [a,b]	122 ± 7 [a]	116 ± 4 [a]	188 ±7 [e]	183 ± 8 [e]	184 ± 1 [e]	178 ± 4 [d,e]	158 ± 7 [c,d]	166 ± 4 [d]	153 ± 10 [c]	179 ± 6 [e]
2-Hexanone	785	58	336 ± 45 [d]	364 ± 16 [d]	300 ± 15 [d]	242 ± 8 [c]	173 ± 8 [b]	165 ± 1 [b]	139 ± 6 [a]	306 ± 8 [d]	304 ± 14 [d]	294 ± 10 [c,d]	295 ± 23 [c,d]	253 ± 15 [c]	218 ± 11 [c]	162 ± 1 [b]	136 ± 8 [a]
Butanoic acid	821	60	-	1187 ± 161 [a]	1954 ± 23 [a,b]	2899 ± 163 [c]	2074 ± 212 [b]	2522 ± 226 [b]	2133 ± 1670 [b]	6087 ± 354 [e]	3176 ± 180 [c,d]	3281 ± 120 [c]	3128 ± 226 [c]	3526 ± 493 [d]	2541 ± 137 [b]	3562 ± 118 [d]	2746 ± 47 [b,c]
2-Heptanone	896	71	485 ± 42 [c]	850 ± 90 [f]	702 ± 85 [e]	364 ± 5 [b]	466 ± 73 [b,c]	57 ± 10 [d]	559 ± 10 [d]	813 ± 196 [e]	762 ± 50 [e,f]	736 ± 43 [e]	584 ± 61 [c,d]	345 ± 27 [b]	584 ± 37 [c,d]	732 ± 26 [e]	212 ± 19 [a]
Sabinene	976	136	-	-	-	-	-	-	-	-	-	-	-	199 ± 22 [a]	272 ± 31 [b]	316 ± 11 [b,c]	374 ± 8 [c]
β-pinene	981	136	-	-	-	40 ± 1 [a]	71 ± 3 [b]	78 ± 2 [b]	86 ± 7 [b]	-	-	-	-	194 ± 7 [c]	24 ± 4 [c]	248 ± 22 [c,d]	290 ± 16
Myrcene	986	136	-	-	-	-	-	-	-	-	-	-	-	196 ± 16 [a]	252 ± 14 [a,b]	318 ± 14 [b]	365 ± 13 [b]
Limonene	1030	136	-	-	-	680 ± 45 [a]	1207 ± 31 [b]	1277 ± 12 [b]	1294 ± 24 [b]	-	-	-	-	-	-	-	-
2-Nonanone	1052	142	36 ± 3 [d]	53 ± 4 [e]	37 ± 5 [d]	30 ± 5 [c]	29 ± 2 [b,c]	21 ± 2 [a]	28 ± 5 [b]	44 ± 1 [e]	29 ± 4 [b]	23 ± 3 [a,b]	33 ± [c d]	32 ± 8 [c]	29 ± 3 [b,c]	31 ± 2 [c]	42 ± 7 [e]
γ-Terpinene	1057	136	-	-	-	100 ± 11 [a]	207 ± 12 [b]	243 ± 5 [b]	23 ± 11 [b]	-	-	-	-	-	-	-	-
Eugenol	1351	164	-	-	-	-	-	-	-	318 ± 2 [a]	1512 ± 66 [b]	2319 ± 70 [b,c]	1800 ± 16 [b,c]	-	-	-	-
Caryophyllene	1428	189	-	-	-	-	-	-	-	1 ± 0 [a]	3 ± 0 [b]	5 ± 0 [c]	7 ± 1 [c]	-	-	-	-
Humulene	1465	93	-	-	-	-	-	-	-	70 ± 4 [a]	174 ± 6 [b]	252 ± 16 [b,c]	246 ± 11 [b,c]	-	-	-	-
Eugenol acetate	1552	164	-	-	-	-	-	-	-	15 ± 1 [a]	37 ± 2 [a]	85 ± 2 [b]	81 ± 2 [b]	-	-	-	-

[1] Kovats' Retention Index on SLB-5 + Supelcowax10 columns; [2] ions used for semi-quantification; [3] not detected. Data are presented as an average relative peak area of triplicates (peak area of the compound divided by peak area of internal standard) with standard deviation value. Data are displayed as mean ± SD.Mean values marked by the different lower-case superscript letters (a–g) within a row denote statistically significant differences ($p < 0.05$) using a two-way ANOVA.

Short-chain fatty acids, such as acetic and butyric (butanoic) acid, were present in all samples fermented with *B. animalis* subsp. *lactis* Bb-12. The levels of both acids did not decrease during the storage of samples for 21 days. These acids can be produced by the lipolysis of milk fat, or they can be specifically formed by the action of LAB or from the deamination of amino acids as products of lactose metabolism or even lipid oxidation. The representative *Bifidobacterium* species used in this study is mainly responsible for producing acetate through the fermentation pathway. In contrast, lactobacilli can produce end products, such as pyruvate by carbohydrate fermentation, during the glycolytic metabolic pathway. The nonfermented samples did not have these acids, which confirms the significant influence of the fermentation process on their formation. The highest increase in the content of acetic and butanoic acids was found in sour cream samples enriched with essential oils at the lowest concentration (12.5%). Volatile organic acids, including acetic and butanoic acids, are important components determining the overall palatability of fermented milk beverages. A significant increase in the content of butyric acid during the storage of products may result in undesirable changes in the smell and taste, characteristic of rancidity. The addition of essential oils to cream subjected to fermentation resulted in the formation of the identified volatile components, which were part of the chemical composition of the used essential oils. Sour cream samples containing lemon peel essential oils contained β-pinene, limonene, and γ-terpinene. On the other hand, samples enriched with clove bud essential oils contained eugenol, eugenol acetate, humulene, and caryophyllene, and those supplemented with juniper berry essential oils contained three terpenes, namely sabinene, β-pinene, and myrcene. The content of these compounds in sour cream samples stored for 21 days increased in proportion to the concentration of essential oils added.

4. Conclusions

The essential oils obtained from lemon peels, clove buds, and juniper berries by the steam distillation method showed antimicrobial activity against the tested strains of *Bifidobacterium* in in vitro conditions. The size of the growth inhibition zones of these bacteria was influenced by both the type of essential oil used and the strain tested. All the analyzed strains of *Bifidobacterium* bacteria were sensitive to clove oil. On the other hand, the largest growth inhibition zones were recorded for the *B. animalis* subsp. *lactis* Bb-12 strain with the use of clove and juniper berry essential oils. However, neither the type of essential oil nor its concentration had any influence on the number of *B. animalis* subsp. *lactis* Bb-12 cells in the samples of cream directly fermented with this strain and those stored for 21 days at 6 °C. The main components of the lemon peel oil were identified to be limonene and γ-terpinene, while the juniper berry essential oil was rich in sabinene and β-myrcene and the clove bud oil was rich in eugenol and eugenol acetate. These compounds were also found among the volatile aroma compounds detected in sour cream samples. The results of this study suggest that the tested essential oils could serve as a natural additive for dairy products, conferring them with additional health-promoting and sensory properties. However, this should be confirmed by further research.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app12031067/s1. Table S1 and Table S1 (continued) entitled "Fatty acid composition (%) of fat extracted from cream, sour cream, and stored sour cream samples enriched with essential oil from lemon peel, clove bud, and juniper berry."

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