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Research Article

Solid-phase micro extraction coupled with GC–MS for determination of the metabolite profile of newly isolated strains of *Lactobacillus delbrueckii ssp. bulgaricus*

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Abstract

The biological and organoleptic properties of Bulgarian yogurt are largely determined by the metabolic activity of the selected strains of *Lactobacillus delbrueckii* ssp. *bulgaricus*. The HS-SPME-GC-MS technique was used to analyze the volatile compounds produced by the following strains: *Lactobacillus delbrueckii* ssp. *bulgaricus* MG1, MG2, MG3 MG4, MG5 MG6, MG7 and *Lactobacillus delbrueckii* ssp. *bulgaricus* MG8. A study to optimize the conditions of SPME was performed. The optimum extraction of the aromatic components was obtained at an extraction temperature of 50°C and a process duration of 50 min. In total, 49 volatile flavor compounds, forming the aromatic profile of the obtained fermented milk, were identified. The production of acetaldehyde, 2,3-butanedione, 2-acetylfuran, 2-nonanone, 2-heptanone and 2-undecanone, pentanoic and octanoic acid, 3-carnene, undecane, dodecane, tridecane and tetradecane was detected for all strains. The only strain that produced acetoin was *Lactobacillus delbrueckii* ssp. *bulgaricus* MG7.

Keywords: headspace solid-phase microextraction, gas chromatography-mass spectrometry, *Lactobacillus delbrueckii* ssp. *bulgaricus*, aroma and flavor compounds, fermented milk

Abbreviations: solid phase microextraction (SPME); gas chromatography-mass spectrometry (GC-MS); Headspace (HS**)**

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Introduction

Lactic acid bacteria (LAB) are industrially important bacteria that are generally used in the fermented food industry, specifically in the manufacture of yogurt (Dan et al. 2017). Yogurt is the most popular fermented milk and is usually produced using mixtures of homofermentative LAB such as *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* as the starter culture (Herve Jimenez et al. 2009; Muramalla and Aryana 2011; Kaneko et al. 2014).
The primary quality characteristics of yogurt include texture, taste, aroma, and flavor (Panagiotidis and Tzia 2001; Pourahmad and Assadi 2005; Sodini et al. 2004). Flavor is one of the most important properties of food products with regard to product acceptability and preference. The sensory properties of dairy products depend largely on the relative balance of flavor compounds derived from fats, protein, or carbohydrates in the milk (Cheng 2010).The distinct flavor of yogurt is a result of lactic acid and a complex mixture of aroma compounds, which include the volatiles already present in the milk and specific compounds produced from milk fermentation (Ott et al. 1997; Tamime and Deeth 1980). More than 90 different volatiles have been identified in yogurt, including carbohydrates, alcohols, aldehydes, ketones, acids, esters, lactones, sulfur-containing compounds, pyrazines, and furan derivatives (Ott et al. 1997).
SPME (solid phase micro extraction) coupled with GC-MS can provide high sensitivity with a small sample volume and canthus be used to analyze the flavor profile of a wide variety of substances (Dan et al. 2017).
In this study, solid-phase micro extraction coupled with GC–MS was used to determine the volatile profiles of eight newly isolated strains *Lactobacillus delbrueckii* ssp. *bulgaricus.*
The aim of this study was to investigate the composition of the volatile compounds in milk that has been fermented by eight newly isolated strains *Lactobacillus delbrueckii* ssp. *bulgaricus*.

Materials and Methods

Microorganisms. Eight strains of lactic acid bacteria of the species *Lactobacillus delbrueckii* ssp. *bulgaricus* were isolated and identified from naturally fermented lactic acid products (yoghurt, cheese, etc.): *Lactobacillus delbrueckii* ssp. *bulgaricus* MG1; *Lactobacillus delbrueckii* ssp. *bulgaricus* MG2; *Lactobacillus delbrueckii* ssp. *bulgaricus* MG3; *Lactobacillus delbrueckii* ssp. *bulgaricus* MG4; *Lactobacillus delbrueckii* ssp. *bulgaricus* MG5; *Lactobacillus delbrueckii* ssp. *bulgaricus* MG6; *Lactobacillus delbrueckii* ssp. *bulgaricus* MG7 and *Lactobacillus delbrueckii* ssp. *bulgaricus* MG8. The selected strains are property of the of "LB-Lact" laboratory in Plovdiv, Bulgaria.

Cultivation of the strains tested. The strains were activated by inoculation in milk 24 h prior to the analysis. Cultivation of the tested strains was carried out under static conditions in sterile, reconstituted (12% dry matter) dry skim milk at 42 ± 1°C. After inoculation, fermentation was allowed to proceed until the pH fell to 4.7. Analysis of volatile compounds was performed immediately after reaching pH 4.7.

HS-SPME-GC-MS analysis. Headspace solid-phase microextraction–gas chromatography-mass spectrometry (HS-SPME-GC-MS) was used to analyze the volatile compounds produced by each of the tested strains *L. delbrueckii* ssp. *bulgaricus.* Toluene (obtained from Sigma-Aldrich) was used as an internal standard. In 20-mL glass vials, 5 mL of sample were mixed with ISTD to a final concentration in each sample of 10 µg/L. The samples were heated at 50°C and subsequently, a SPME fiber (50/30 um DVB/Carboxen/PDMS; Supelco) was exposed in the headspace for 50 minutes. The fiber was then immediately inserted into the injection port of a Trace 1300 GC (Thermo Fisher Scientific) for 5 min at 270°C to desorb volatile compounds into the GC. The optimum extraction conditions were selected based on preliminary experiments on SPME extraction of samples of *L. delbrueckii* ssp. *bulgaricus* MG1 at different extraction temperatures (40°C, 50°C, 60°C, and 70°C) and at different time intervals (30, 40, 50 and 60 min).

Metabolites identification. The volatile compounds from each combination were identified using a Trace 1300 GC equipped with a ISQ QD, Single Quadrupole Mass Spectrometer (both Thermo Fisher Scientific) equipped with an TR-5MS column (length, 30 m; i.d., 0.25 mm; film thickness, 0.25 µm; Thermo Fisher Scientific).

Helium was used as the carrier gas at 1 mL/min. The GC temperature was initially maintained at 35°C for 5 min and then increased to 140°C at a rate of 4°C/min for 5 min, heated to 270°C at a rate of 10°C/min, and, finally, held at 270°C for 5 min. The mass detection was made according to the manufacturer's recommendations in the full scan mode. The ion source and transfer line temperatures were 220°C and 260°C, respectively. The mass spectra from each sample were recorded using a scan range of 40-400 m/z. Each sample measurement was carried out in triplicate.

Results and Discussion

Extraction temperature and time optimization. A preliminary experiment to optimize extraction conditions, including the sample temperature and extraction time of volatile compounds present in samples, was performed using SPME fiber (50/30 µm DVB/Carboxen/PDMS). The sample temperature and extraction time are important parameters in the SPME sampling process and can increase extraction efficiency when optimized. The sample temperature and extraction time are discussed for *L. delbrueckii* ssp. *bulgaricus* MG1. Extraction was compared at temperatures of 40, 50, 60 and 70°C. The relative peak areas were enhanced at temperatures up to 50°C and then began to decline (Fig. 1).Thus, a temperature of 50°C was used to study extraction time. Using extraction times ranging from 30 to 60 min, the relative peak areas were enhanced as time increased up to 50 min and then began to decline (Figure 2). The optimum extraction conditions were 50°C for 50 min.

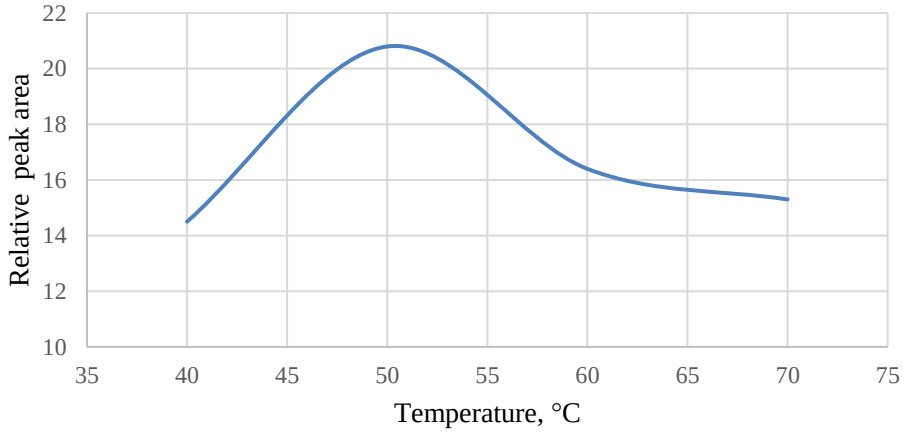

Figure 1. Effects of extraction temperature on extraction efficiency

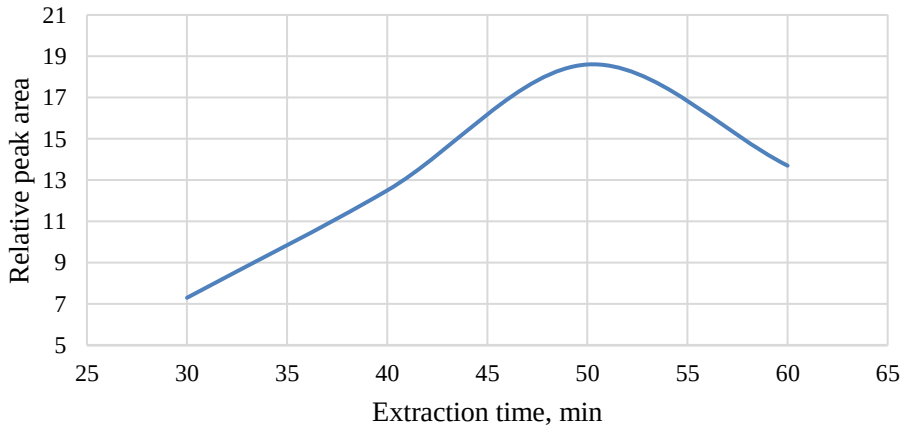

Figure 2. Effects of extraction time on extraction efficiency

The aromatic components biosynthesized by the studied strains were determined by Headspace solid-phase microextraction–gas chromatography-mass spectrometry. A total of 49 aromatic compounds (presented in Table 1) were identified. The aromatic components are grouped into separate classes depending on their chemical structure.

Aldehyde compounds. The main aromatic component representative of the aldehydes is acetaldehyde, which is produced by all studied strains, but in different amounts. The highest level of acetaldehyde was detected for strain *Lactobacillus delbrueckii* ssp. *bulgaricus* MG7. For this strain, the relative peak area was the largest – 7.5000, followed by strain *Lactobacillus delbrueckii* ssp. *bulgaricus* MG8 – 4.5157. Strains *Lactobacillus delbrueckii* ssp. *bulgaricus* MG3, MG4, MG5 and MG6 occupied an intermediate position with respect to the amount of produced acetaldehyde. Comparable values of the relative area of the acetaldehyde peaks were observed – 0.4884, 2.9115, 2.5463 and 2.3063 for the respective strains. Significantly lower concentrations of acetaldehyde were observed for strains *Lactobacillus delbrueckii* ssp. *bulgaricus* MG1 and MG2 with relative peak areas of 1.2465 and 1.7563 respectively. All tested strains also produced heptanal, and the relative area of the heptanal peaks for the different strains varied from 0.0085 to 0.0343. Furaldehyde was detected only for strains *Lactobacillus delbrueckii* ssp. *bulgaricus* MG4, MG5, MG7 and MG8 with relative peak areas in the range of 0.0539 and 0.4155 for the relative strains (Table 1). Except for strain *Lactobacillus delbrueckii* ssp. *bulgaricus* MG3, all the others produced 3-Hydroxybutanal, as the relative peak areas for this compound were in the range 0.0260 to 0.0405 for the different strains. Benzaldehyde was found only in yogurt obtained with strains *Lactobacillus delbrueckii* ssp. *bulgaricus* MG1, MG2 and MG3. The relative peak areas for this component were close together (in the range of 0.0445 to 0.0498), indicating a comparable amount of benzaldehyde for these strains. In the yogurt obtained with strains *Lactobacillus delbrueckii* ssp. *bulgaricus* MG2, MG3, MG4, MG5, MG6 and MG7, benzacetaldehyde was detected with a relative peak area for the respective strains in the range from 0.0068 to 0.0606. Nonanal was produced by all the strains excluding *Lactobacillus delbrueckii* ssp. *bulgaricus* MG7, and the relative peak area varied from 0.0075 to 0.0319. Ethyl benzaldehyde was found for *Lactobacillus delbrueckii* ssp. *bulgaricus* MG1, MG2, MG3, MG4 and MG5 with a relative peak area in the range of 0.0062 to 0.0113; however, this aromatic component was not detected for the other three strains. 2-octanal was detected in all the strains without *Lactobacillus delbrueckii* ssp. *bulgaricus* MG3. Hexanal was missing only for *Lactobacillus delbrueckii* ssp. *bulgaricus* MG2. Decanal was identified only in *Lactobacillus delbrueckii* ssp. *bulgaricus* MG1, and none of the other strains produced it.

Ketone compounds. The main aromatic component in yogurt from the group of ketones is acetoin. Of all the strains tested, only *Lactobacillus delbrueckii* ssp. *bulgaricus* MG7 produced acetoin, with a relative peak area for this component of 2.2933. All strains tested produced 2,3-butanedione, 2-acetylfuran, 2-nonanone, 2-heptanone and 2-undicanone. The relative peak areas for these aromatics varied in the range of 0.0232 to 1.0444 for the respective strains. 2,5-dimethyl-4-hydroxy-3 (2H) -furanone was detected only in the *Lactobacillus delbrueckii* ssp. *bulgaricus* MG3 with a relative peak area of 0.0164. Strains *Lactobacillus delbrueckii* ssp. *bulgaricus* MG1, MG2 and MG4, synthesized 1-butanone in comparable concentrations, confirmed by the values of to the relative peak areas: 0.0171, 0.0163 and 0.0162, respectively. A lower value of 1-butanone for strain *Lactobacillus delbrueckii* ssp. *bulgaricus* MG6, was observed due to the smaller relative peak area for this component - 0.0080. The aromatic component 5-methyl-3-heptanone was found for only three of the studied strains - *Lactobacillus delbrueckii* ssp. *bulgaricus* MG1, MG4 and MG7. The concentration of 5-methyl-3-heptanone was highest for MG7 (relative peak area 0.0136), while for the other two strains, the concentrations of this component were similar (relative peak area - 0.0089 and 0.0088). 3-methyl-2-butanone was produced by all of the strains excluding *Lactobacillus delbrueckii* ssp. *bulgaricus* MG7. For all of the other strains, the relative peak areas for this aromatic component varied in the range of 0.0150 to 0.0343. 2',4'-Dimethoxyacetophenone was

detected in only two of the studied strains - *Lactobacillus delbrueckii* ssp. *bulgaricus* MG1 and MG6.The amount of the metabolite was higher in yogurt obtained with MG1 due to the larger relative peak area - 0.0725 compared to strain MG6 with a relative peak area of 0.0551.

Acid compounds. The content of organic acids related to the aromatic profile of yoghurts obtained with monocultures from different strains of *Lactobacillus delbrueckii* ssp. *bulgaricus* was determined. The studies have shown that all strains produce Pentanoic and Octanoic acid. For these acids, the relative area of the peaks for the different strains varies in the range from 0.0096 to 0.1363. Butanoic acid is synthesized from strains *Lactobacillus delbrueckii* ssp. *bulgaricus* MG1 to MG7. This acid was not detected in MG8. Larger and comparable amounts of butanoic acid were detected for strains MG1 and MG7, which had larger and similar values of the relative peak area - 0.0122 and 0.0126. Strains MG2, MG3 and MG4 occupied an intermediate position in terms of the amount of butanoic acid synthesized. They also had similar metabolite values due to close relative peak areas - 0.0092, 0.0082 and 0.0086. Strains MG5 and MG6 produced the lowest concentrations of butanoic acid, with relative peak areas for this component being 0.0066 and 0.0055, respectively.

Formic acid was detected for only one strain - *Lactobacillus delbrueckii ssp. bulgaricus* MG3 with a relative peak area of 0.4647. One of the primary organic acids influencing the aromatic profile was acetic acid. The highest concentration of acetic acid was determined in yogurt obtained with strain *Lactobacillus delbrueckii* ssp. *bulgaricus* MG1. For this strain, the relative peak area of this metabolite was 1.1500. Strains MG2, MG4, MG7 and MG8 produced acetic acid in lower and closer concentrations, as the values of their relative peak areas were also close at 0.2114, 0.2337, 0.2441 and 0.2186, respectively. The lowest concentration of acetic acid was detected for strain MG5, where the relative peak area was 0.1334. Hexanoic acid in a relatively high concentration was detected only for strain *Lactobacillus delbrueckii* ssp. *bulgaricus* MG3, with a relative peak area of 0.7076.

Alcohol compounds. In total, 6 alcoholic compounds involved in the formation of the aromatic profile of the obtained fermented milks were identified. The compound that was detected in the largest amount was 2-furanmethanol. The highest detected concentration of this metabolite was for strains of *Lactobacillus delbrueckii* ssp. *bulgaricus* MG3 and MG7 with relative peak areas of 3.9455 and 3.5246, followed by strains MG4 and MG8 with relative peak areas - 2.4485 and 2.5446. The lowest concentrations of 2-furanmethanol were detected for strains MG5 and MG6, with relative peak areas for this metabolite of 1.2833 and 1.5184, respectively. This metabolite was not detected for strains MG1 and MG2.

2-ethylhexanol was detected for strains *Lactobacillus delbrueckii* ssp. *bulgaricus* MG1, MG2, MG3, MG4, MG5, MG6 and MG8 and was produced in different concentrations by the respective strains. The relative peak area for this metabolite for the different strains varied from 0.0092 to 0.02640. For strain MG7, 2-ethylhexanol was not detected. The presence of 3-methyl-butnol was detected in milk fermented with strains *Lactobacillus delbrueckii* ssp. *bulgaricus* MG1, MG2, MG4, MG5 and MG7 with relative peak areas varying in the range of 0.0039 to 0.0160 for the respective strains. However, 3-methyl-butnol was not detected in MG3, MG6 and MG8. 2-nonanol was detected for strains *Lactobacillus delbrueckii* ssp. *bulgaricus* MG1, MG2, MG3, MG4, MG7 and MG8. The relative peak areas for the respective strains varied from 0.0172 to 0.2890. For strains MG5 and MG6, 2-nonanol was not detected. 2-undecanol was detected in strains *Lactobacillus delbrueckii* ssp. *bulgaricus* MG1, MG2, MG4, MG5 and MG7. The relative peak areas for these strains varied from 0.0038 to 0.0477. 2-undecanol was not detected in strains MG3, MG6 and MG8.3-methylbutanol was found only in yogurts obtained with *Lactobacillus delbrueckii* ssp. *bulgaricus* MG1, MG4 and MG7. The lowest relative area (0.0032), respectively the lowest concentration of this metabolite was observed for strain MG4.In the other two strains, the relative peak areas for this component were 0.0083 and 0.0070, respectively. 3-methylbutanol was not detected for strains MG2, MG3, MG5, MG6 and MG8.

Ester compounds. Four ester compounds that influence the aromatic profile of fermented milk were detected and identified.

Methyl N-hydroxybenzenecarboximidoate was the compound detected in the highest concentration of ester compounds, but it was produced by only two strains - *Lactobacillus delbrueckii* ssp. *bulgaricus* MG1 and MG2 with relative peak areas of 1.0508 and 0.7181, respectively. This metabolite was not identified in other samples. 8-Tetradecyn-1-ol acetate was detected in different concentrations in fermented milk with strains *Lactobacillus delbrueckii* ssp. *bulgaricus* MG1, MG2, MG3, MG4, MG5, MG6 and MG8. The relative peak areas for this compound varied from 0.0133 to 0.0595, and the compound was only absent in strain *Lactobacillus delbrueckii* ssp. *bulgaricus* MG7.

3-Butanoic acid, 2-oxo-4-phenyl-, methyl ester in different concentrations was detected for strains *Lactobacillus delbrueckii* ssp. *bulgaricus* MG1, MG3, MG4, MG5 and MG6. The relative areas of the peaks for this compound varied from 0.0038 to 0.0176 for the respective strains. However, this metabolic product was absent for strains MG2, MG7 and MG8. 4-ethylbenzoic acid, methyl ester in different concentrations was observed in all strains except *Lactobacillus delbrueckii* ssp. *bulgaricus* MG3, and the relative peak areas for this metabolite varied from 0.0053 to 0.0497.

Aromatic hydrocarbons. All tested strains produced 3-carene, undecane, dodecane, tridecane and tetradecane in different concentrations. For these metabolites, the relative peak areas varied from 0.0077 to 0.1752 for the respective strains. The studied strains differed in their ability to produce other aromatic hydrocarbons. 2-methylundecane was produced only by strains *Lactobacillus delbrueckii* ssp. *bulgaricus* MG1, MG2, MG4, MG5 and MG6. The relative area of the peaks for this compound varied from 0.0052 to 0.0105. However, this metabolite was not detected for MG3, MG7 and MG8. 2,4,6-trimethyldecane was detected at different concentrations in fermented milk with *Lactobacillus delbrueckii* ssp. *bulgaricus* MG1, MG3, MG4, MG5 and MG6 (relative peak areas ranging from 0.0053 to 0.0092 for the respective strains) but was not detected in strains MG2, MG7 and MG8. Pentadecane at different concentrations was identified for strains Lactobacillus delbrueckii ssp. bulgaricus MG1, MG2, MG4, MG5, MG6, MG7 and MG8, with the relative peak areas for this metabolite varying from 0.0125 to 0.0357. Lactobacillus delbrueckii ssp. bulgaricus MG3 was the only strain in which pentadecane was not detected. Hexadecane was not detected in fermented milk with strains of Lactobacillus delbrueckii ssp. bulgaricus MG1 and MG8. However, the other studied strains produced hexadecane in different concentrations. The relative peak area for this metabolite varied in the range of 0.0036 to 0.0126. 2,3,5,8-tetramethyl decane was detected only for strains Lactobacillus delbrueckii ssp. bulgaricus MG1, MG2 and MG3 in similar concentrations, and the relative peak areas for this metabolite were comparable for the individual strains - 0.0068, 0.0062 and 0.0063.

Conclusions

Our results showed that different strains of *Lactobacillus delbrueckii* ssp. *bulgaricus*, produce different aromatic components with a total of 49 aromatic compounds that were identified in this study. The aromatic compounds were grouped into separate classes (aldehydes, ketones, organic acids, esters, alcohols, aromatic hydrocarbons) based on their chemical structure. Therefore more work should be directed toward a more comprehensive understanding of mechanisms involved in the production of the critical aromatic compounds as related to human health in order to select strains the can be considered as probiotic strains of *Lactobacillus delbrueckii* ssp. *bulgaricus.*

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Table 1. Volatile compounds produced by different strains *L. delbrueckii* ssp. *bulgaricus*

№	Volatile Compound	Chemical formula	RT (min)	Metabolites produced by strains *L. delbrueckii ssp. bulgaricus**							
				MG1	MG2	MG3	MG4	MG5	MG6	MG7	MG8
			Aldehyde Compounds								
1	Acetaldehide	C_2H_4O	1.89	1.2465	1.7563	2.4884	2.9115	2.5463	2.3063	7.5000	4.5157
2	Furaldehyde	$C_5H_4O_2$	9.53	-	-	-	0.0593	0.2069	-	0.4002	0.4155
3	3-Hydroxybutanal	$C_4H_8O_2$	13.23	0.0287	0.0260	-	0.0358	0.0402	0.0311	0.0405	0.0333
4	Benzaldehyde	C_7H_6O	15.22	0.0445	0.0494	0.0498	-	-	-	-	-
5	Benzacealdehyde	C_8H_8O	18.65	-	0.0068	0.0606	0.0174	0.0130	0.0083	0.0231	-
6	Nonanal	$C_9H_{18}O$	20.88	0.0102	0.0075	0.0319	0.0171	0.0081	0.0076	-	0.0077
7	Ethylbenzaldehyde	$C_9H_{10}O$	23.97	0.0113	0.0101	0.0162	0.0116	0.0062	-	-	-
8	Heptanal	$C_7H_{14}O$	26.37	0.0302	0.0319	0.0343	0.0251	0.0161	0.0129	0.0116	0.0085
9	2-Octenal	$C_8H_{14}O$	29.3	0.0730	0.0103	-	0.0441	0.0109	0.0159	0.1417	0.0636
10	Hexanal	$C_6H_{12}O$	33.94	0.0626	-	0.0747	0.0809	0.0233	0.0338	0.2214	0.1374
11	Decanal	$C_{10}H_{20}O$	40.91	0.0127	-	-	-	-	-	-	-
			Ketone Compounds								
12	Acetoin	$C_4H_8O_2$	4.89	-	-	-	-	-	-	2.2933	-
13	2,3-Butanedione	$C_4H_6O_2$	11.86	0.4694	0.2654	0.6575	0.6595	0.2700	0.2376	0.3588	0.4247
14	2-Acetylfuran	$C_6H_6O_2$	12.88	0.0431	0.0593	0.0837	0.0505	0.0294	0.0252	0.0477	0.0424
15	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	$C_6H_8O_3$	14.6	-	-	0.0164	-	-	-	-	-
16	1-Butanone, 1-phenyl-	$C_{10}H_{12}O$	17.27	0.0171	0.0163	-	0.0162	-	0.0080	-	-
17	2-nonanone	$C_9H_{18}O$	20.3	0.6354	0.2783	1.0381	1.0444	0.3099	0.3139	0.2236	0.5183
18	2-heptanone	$C_7H_{14}O$	24.13	0.0384	0.0404	0.0522	0.0395	0.0247	0.0187	0.0374	0.0232

№	Volatile Compound	Chemical formula	RT (min)	Metabolites produced by strains *L. delbrueckii* ssp. *bulgaricus**							
				MG1	MG2	MG3	MG4	MG5	MG6	MG7	MG8
19	5-Methyl-3-heptanone	$C_8H_{16}O$	26.88	0.0089	-	-	0.0088	-	-	0.0136	-
20	3-Methyl-2-butanone	$C_5H_{10}O$	27.43	0.0559	0.0150	0.0703	0.0478	0.0177	0.0179	-	0.0343
21	2-Undecanone	$C_{11}H_{22}O$	27.73	0.1362	0.0922	0.1892	0.1912	0.0637	0.0577	0.1463	0.1034
22	2',4'-Dimethoxyacetophenone	$C_{10}H_{12}O_3$	29.6	0.0725	-	-	-	-	0.0551	-	-
			Acid Compounds								
23	Formic acid	CH_2O_2	3.79	-	-	0.4647	-	-	-	-	-
24	Acetic acid	$C_2H_4O_2$	14.21	1.1500	0.2114	-	0.2337	0.1334	-	0.2441	0.2186
25	Hexanoic acid	$C_6H_{12}O_2$	15.88	-	-	0.7076	-	-	-	-	-
26	Pentanoic acid	$C_5H_{10}O_2$	16.35	0.0163	0.0200	0.0281	0.0316	0.0193	0.0136	0.0100	0.0096
27	Butanoic acid	$C_4H_8O_2$	20.58	0.0122	0.0092	0.0082	0.0086	0.0066	0.0055	0.0126	-
28	Octanoic acid	$C_8H_{16}O_2$	23.29	0.1098	0.0426	0.3077	0.1363	0.0582	0.0326	0.0377	0.0307
			Alcohol Compounds								
29	2-Furanmethanol	$C_5H_6O_2$	10.32	-	-	3.9455	2.4485	1.2833	1.5184	3.5246	2.5446
30	2-Ethylhexanol	$C_8H_{18}O$	17.76	0.0105	0.0264	0.0215	0.0203	0.0133	0.0092	-	0.0110
31	3-Methyl-2-butanol	$C_5H_{12}O$	20.06	0.0139	0.0124	-	0.0160	0.0039	-	0.0140	-
32	2-Nonanol	$C_9H_{20}O$	20.7	0.0985	0.0330	0.1843	0.2890	-	-	0.0172	0.0189
33	2-Undecanol	$C_{11}H_{24}O$	28.04	0.0262	0.0114	-	0.0477	0.0038	-	0.0231	-
34	3-Methylbutanol	$C_5H_{12}O$	31.56	0.0083	-	-	0.0032	-	-	0.0070	-

№	Volatile Compound	Chemical formula	RT (min)	Metabolites produced by strains *L. delbrueckii* ssp. *bulgaricus**							
				MG1	MG2	MG3	MG4	MG5	MG6	MG7	MG8
			Ester Compounds								
35	Methyl N-hydroxybenzenecarboximidoate	$C_8H_9NO_2$	12.16	1.0508	0.7181	-	-	-	-	-	-
36	8-Tetradecyn-1-ol acetate	$C_{16}H_{28}O_2$	27.61	0.0319	0.0225	0.0595	0.0243	0.0113	0.0067	-	0.0200
37	3-Butanoic acid, 2-oxo-4-phenyl-, methyl ester	$C_{12}H_{18}O_2$	28.48	0.0094	-	0.0176	0.0102	0.0043	0.0038	-	-
38	4-Ethylbenzoic acid, methyl ester	$C_{10}H_{12}O_2$	29.79	0.0254	0.0233	-	0.0497	0.0053	0.0222	0.0218	0.1245
			Aromatic Hydrocarbons								
39	3-Carene	$C_{10}H_{16}$	13.45	0.1117	0.1087	0.1713	0.1752	0.0511	0.0487	0.0658	0.0643
40	Undecane	$C_{11}H_{24}$	18.52	0.0294	0.0212	0.0466	0.0437	0.0356	0.0210	0.0204	0.0117
41	Dodecane	$C_{12}H_{26}$	24.22	0.0444	0.0512	0.0603	0.0593	0.0312	0.0280	0.1012	0.0703
42	Tridecane	$C_{13}H_{28}$	26.76	0.0181	0.0177	0.0549	0.0283	0.0178	0.0116	0.0110	0.0077
43	2-Methylundecane	$C_{12}H_{26}$	28.39	0.0062	0.0066	-	0.0105	0.0066	0.0051	-	-
44	Tetradecane	$C_{14}H_{30}$	31.12	0.1163	0.1368	0.1647	0.1336	0.0812	0.0696	0.1415	0.1115
45	2,4,6-Trimethyldecane	$C_{13}H_{28}$	34.21	0.0092	-	0.0090	0.0087	0.0053	0.0075	-	-
46	Pentadecane	$C_{15}H_{32}$	35.02	0.0357	0.0309	-	0.0309	0.0148	0.0125	0.0166	0.0151
47	Hexadecane	$C_{16}H_{34}$	39.02	-	0.0126	0.0125	0.0065	0.0040	0.0036	-	0.0069
48	Octadecane	$C_{18}H_{38}$	41.1	0.0061	0.0045	0.0047	0.0066	0.0025	0.0028	0.0043	0.0057
49	2,3,5,8-Tetramethyldecane	$C_{14}H_{30}$	41.25	0.0068	0.0062	0.0063	-	-	-	-	-

*Relative Area to the Peak Area of the Internal Standard

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