Chemical Compositions and Aroma Evaluation of Volatile Oil from the Industrial Cultivation Medium of Enterococcus faecalis

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Abstract: Enterococcus faecalis is one of the major lactic acid bacterium (LAB) species colonizing the intestines of animals and humans. The characteristic odor of the volatile oils obtained from both the liquid medium after incubation (MAI) and liquid medium before incubation (MBI) in the cultivation process of E. faecalis was investigated to determine the utility of the liquid medium. In total, fifty-six and thirty-two compounds were detected in the volatile oils from the MAI (MAI oil) and MBI (MBI oil), respectively. The principle components of MAI oil were 2,5-dimethylpyrazine (19.3%), phenylacetaldehyde (19.3%), and phenylethyl alcohol (9.3%). The aroma extract dilution analysis (AEDA) method was performed using gas chromatography-olfactometry (GC-O). The total number of aroma-active compounds identified in the volatile oil from MBI and MAI was thirteen compounds; in particular, 5-methyl-2-furanmethanol, phenylacetaldehyde, and phenylethyl alcohol were the most primary aroma-active compounds in MAI oil. These results imply that the industrial cultivation medium after incubation of E. faecalis may be utilized as a source of volatile oils.

Key words: lactic acid bacterium (LAB), Enterococcus faecalis, utilizing liquid medium, media after incubation (MAI), aroma extract dilution analysis (AEDA)

1 INTRODUCTION

Liquid culture medium, which is indispensable for the cultivation of bacteria such as lactic acid bacteria (LAB), is used for probiotics. Probiotics are added to foods and dietary supplements to improve the health of humans and animals¹. Probiotics are microorganisms that, among other things, constitute a protective barrier against pathogenic microflora stimulate the immune system, improve the nutritive value of food, reduce cholesterol levels and facilitate lactose hydrolysis. The most important probiotic is LAB, which are used as a starter cultures for the fermentation of foods owing to their contribution to aroma and flavor development⁶. Bacterial of the genus Enterococcus belong to LAB, and E. faecalis is one of the major LAB species colonizing the intestines of animals and humans. E. faecalis is known to have highly immunogenic and cholesterol-lowering action, and has been used as a countermeasure to prevent pollinosis⁴. However, the cultivation process for LAB (e.g., E. faecalis) yields large amounts of liquid medium after incubation (MAI) of the microorganism. Currently, the practical use of MAI is limited: however, MAI is thought to contain volatile and biological compounds with potential applications. Therefore, as an initial step toward utilizing the liquid medium produced by cultivation processes, we focused on determining the composition and identifying the characteristic aroma-active compounds in the volatile components of the MAI. It is claimed that the volatile oil components are potentially useful for multiple purposes, including the perfume industry.

Interestingly, for cultures of E. faecalis, which is a heterofermentative LAB, a remarkable difference exists between the odor of the liquid medium before incubation (MBI) and the MAI. This difference occurs because heterofermentative LAB produce a typical flavor and aroma-ac-
tive compounds, in addition to the primary lactic acid product, during fermentation of foods\textsuperscript{5,6}. However, there are no detailed reports on the aroma-active compounds in the MAI of \textit{E. faecalis}.

In flavor analysis, gas chromatography-olfactometry (GC-O) is the most used method for the evaluation of odorants; in particular, GC-O including aroma extract dilution analysis (AEDA) has been a useful method for estimating the contribution of the key aroma-active compounds. By means of sniffing serial dilutions of the volatile oil, the volatile components can be ranked according to odor potency\textsuperscript{7}. The odor potency is expressed as the flavor dilution factor (FD) factor. The FD factor is the ratio of the concentration of a component in the initial concentration to the most diluted concentration in which the odor could be detected by GC-O. The significant contribution of each odorant to the characteristic flavor can be determined by the odor active value (OAV). The OAV is the ratio of the concentration to the odor threshold of the component, it is well accepted that components with high OAV contribute more to the aroma of food.

The aim of this initial study was to investigate the chemical composition of and characteristic aroma-active compounds present in MAI oil in comparison with those found in MBI oil using the AEDA method and the concept of OAV, as a first step toward utilizing the liquid medium.

2 EXPERIMENTAL PROCEDURES

2.1 Materials

The \textit{E. faecalis} medium was prepared as described by Sahasrabudhe and Pathade\textsuperscript{8}. The MAI was prepared by filtering the bacterial cells of \textit{E. faecalis} after incubation for 24 h. The MBI (blank sample), prepared under identical conditions except for the cultivation of bacterial cells, was contributed by Nitto Pharmaceutical Industries, Ltd. in September 2013.

2.2 Extract of the volatile oil using hydrodistillation

The MAI or MBI (1.5 kg) was hydrodistilled with a Likens-Nickerson-type apparatus (Osaka Rikou, Osaka, Japan). The volatile oil obtained from the MAI or MBI was dried over anhydrous sodium sulfate followed by dilution with diethyl ether to a concentration of 10 mg/mL for analysis by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The yields of the volatile oils were as follows: MAI oil, 53 mg/1.5 kg sample (0.004%); MBI oil, 57 mg/1.5 kg sample (0.004%). The volatile oils were kept in sealed glass vials and stored at 4°C in a freezer prior to analysis.

2.3 Gas chromatography (GC)

The GC analysis was performed using an Agilent Technologies-6890N gas chromatograph equipped with a flame ionization detector (FID). The volatile oil was analyzed on an HP-5MS column (5% phenyl 95% polydimethylsiloxane, 30 m × 0.25 mm i.d. 0.25 μm film thickness). Helium was used as the carrier gas at a flow rate of 1.8 mL/min, and 1 μL of sample was injected at a split ratio of 1:40. The oven temperature was programmed to increase from 40°C to 260°C at a rate of 4°C/min finishing with a 5 min hold time. The temperatures of the injector and detector were 270°C and 280°C, respectively.

2.4 Gas chromatography-mass spectrometry (GC-MS)

The GC-MS analysis was carried out using an Agilent Technologies 6890N gas chromatograph, coupled to an Agilent 5973 MSD mass spectrometer. An HP-5MS column (5% phenyl-95% polydimethylsiloxane, 30 m × 0.25 mm i.d. 0.25 μm film thickness) was used. The temperatures of the oven, injector, and detector were the same as those adopted above for the GC analysis. Helium was used as the carrier gas at a flow rate of 1.8 mL/min. One μL of sample was injected at a split ratio of 1:40. The electron impact (EI) energy was 70 eV, and the ion source temperature was set at 230°C. The spectrum was measured from \textit{m/z} 39 to 450 at a rate of 2.39 scans/s.

2.5 Sniffing test

A trained panel of sensory evaluation specialists measured the odor intensities of the main aromatic constituents of both oils. Ten panelists, aged 21 to 55 years (8 males and 2 females, members of Kinki University, Japan), participated in this study. Sensory-analysis sessions were performed only after suitable training (>30 h). A sniffing test by GC-O was carried out using an Agilent Technologies-6890N gas chromatograph equipped with an Agilent 5973 MSD mass spectrometer and sniffing port ODP 2 (Office Detector Port 2, Gerstel). The GC was equipped with an HP-5MS column (5% phenyl 95% polydimethylsiloxane, 30 m × 0.25 mm i.d. 0.25 μm film thickness). The sample was injected into the GC in splitless mode. The GC effluent from the capillary column was split 1:1 (v/v) between the mass spectrometer and the sniffing port. The oven conditions, injector and detector temperature, the carrier gas, flow rate, and ionization mode were the same as those described above for GC-MS.

2.6 Identification of components

The volatile components of the MBI and MAI were identified by comparing their retention indices (RI) and mass spectra with published data\textsuperscript{9}, previous literature studies\textsuperscript{30-36}, digital libraries (Mass Finder 4 and NIST 02), and Aroma office version 3.0 (Nishikawa Keisoku Co. Ltd.), which includes 72,120 RI entries of aroma components from literature sources. The RI values were calculated for a homologous series of \textit{n}-alkanes (C\textsubscript{7}-C\textsubscript{30}) using an HP-5MS column.
Aroma evaluation of the cultivation medium of Enterococcus faecalis

2.7 Quantification of components

The quantitative analysis was performed by means of the internal standard addition method (alkanes C₈ and C₁₉). The volatile oil or aroma extract was diluted by a factor of 100 using diethyl ether to achieve a 1 mL volume, followed by the addition of 4 µL of a C₈ and C₁₉ mixed solution (1 mg/mL) to the diluted oil. These samples were subjected to GC-MS and GC-FID determinations. The quantitative composition of the oil was determined using GC-FID by assuming the total amount of detected components in the oil to be 100%. The quantitative analysis of the characteristic odor compounds of the oil was performed using the percentage peak areas obtained using GC–FID. The quantity of each component was obtained by integrating the peak areas of the chromatogram without correction factors. All determinations were performed in duplicate and averaged.

2.8 Aroma extract dilution analysis (AEDA)

The highest sample concentration (10 mg/mL) was assigned a FD factor of one. The volatile oil was diluted stepwise with diethyl ether (1 + 1, v/v), and aliquots of the dilutions (1 µL) were evaluated. Ten trained panelists sniffed the aromas isolated by GC-O on the HP-5MS capillary column. For each component, the result was expressed as the FD factor, which is the ratio of the concentration of the odorant in the initial volatile oil to its concentration in the most diluted volatile oil whose the odor is still detectable by GC-O.

2.9 Determination of odor activity value (OAV)

The OAV were determined by dividing the concentration of each component by its odor threshold. The odor threshold data were obtained from reported literature data.

3 RESULTS AND DISCUSSION

3.1 Chemical constituents of the two oils

The MBI and MAI of E. faecalis furnished pale yellowish oils in yields of 0.0035% and 0.0038% (w/w), respectively. The volatile oil obtained from the MBI oil had a sweet-green odor, and that from the MAI oil had a sweet-spicy odor. In the two oils, an impressive total of seventy-five compounds were identified, which comprised 100.0% of the two oils (Table 1).

In the MBI oil (blank sample), phenylacetaldehyde (peak 9, 15.3%) was the main compound, followed by 9-octadecenamide (peak 72, 8.4%) and eicosane (peak 59, 8.2%). The classification of the oil on the basis of the predominant functional group (Table 2) is summarized as follows: ten aldehydes (33.6%; seven aliphatic 15.4% and three aromatic 18.3%), nine hydrocarbons (31.6%; eight aliphatic 29.8% and one aromatic 1.8%), seven N-containing compounds (21.8%; two aliphatic 11.1% and five aromatic 10.7%), one ester (1.5%; aliphatic 1.5%), one ether (1.5%; aliphatic 1.5%), and four unknown (10.0%).

In the MAI oil, 2,5-dimethylpyrazine (peak 4, 19.3%) and phenylacetaldehyde (peak 9, 19.3%) were the main compounds, followed by phenylethyl alcohol (peak 14, 9.3%). The classification of the oil on the basis of the predominant functional group (Table 2) is summarized as follows: nine aldehydes (30.1%; five aliphatic 7.7% and four aromatic 22.4%), eight N-containing compounds (29.1%; one aliphatic 0.4% and seven aromatic 28.7%), seven alcohols (21.6%; seven aromatic 21.6%), nineteen hydrocarbons (11.0%; sixteen aliphatic 9.2% and three aromatic 1.8%), three ketones (2.1%; three aromatic), one ester (0.6%; aliphatic), and eight unknown (5.5%) in Table 2.

Table 2 shows a comparison of the classes and percentages of the components in the two oils. The MBI oil efficiently furnished aldehydes, hydrocarbons, and N-containing compounds, which are the three classes with relatively high contents in the oil. The MAI oil had a relatively high percentage of aldehydes, N-containing compounds, and alcohols. Only seven alcohols were detected in the MAI oil, including 2-furanmethanol, 5-methyl-2-furanmethanol, phenyl alcohol, phenylethyl alcohol, chavicol, 2,4-di-tert-butylbenzoquinone, and 2,4-di-tert-butylphenol. Furthermore, all the produced alcohols were primary alcohols. On the other hand, the amount of hydrocarbons in MAI was less than half of that in MBI, and it is suggested that hydrocarbons were converted to alcohols by E. faecalis. Of the N-containing compounds in MAI, 95.2% were pyrazine compounds. Moreover, four pyrazine compounds (2-methylpyrazine, 2,5-dimethylpyrazine, 2-ethyl-5-methylpyrazine, and 3-ethyl-2,5-dimethylpyrazine) were identified in the MAI oil were not detected in the MBI oil. These pyrazine compounds are important flavor components in MAI, and may be synthesized by microorganisms. Pyrazines, which are aromatic heterocyclic nitrogen-containing compounds, and their analogues (e.g., alkylated pyrazines) are produced by some microorganisms, and possess a great variety of odors. For this reason, these components have been employed as flavor and taste enhancers in the food industry. Pyrazines are responsible for the aroma of various foods including beans, nuts, meat, potatoes, and coffee. In particular, 2,5-dimethylpyrazine which was identified in the MAI oil, is used as a flavoring agent in breakfast cereal. In comparison with L. acidophilus and L. brevis which we reported previously, a significant amounts of 2,5-dimethylpyrazine was produced by E. faecalis. Thus, it is thought that MAI oil from E. faecalis could be a method to easily obtain 2,5-dimethylpyrazine in industry. In a study to elucidate the mechanism of production of pyrazine by the bacteria, a mutant of Corynebacterium glutamicum, in which one enzyme of the isoleucine metabolic pathway suffered a loss, was used. In five days, 3 g/L of pyrazines were produced, and then, acetoin was produced through
acetolactic acid by pyruvic acid. It was reported that pyrazines formed from ammonia and acetoin \(^42\). In addition, pyrazines are known to be chemically formed by an aminocarbonyl reaction in the presence of a related material. It was reported that a substantial amount of pyrazines was generated at pH 6.88 (22°C, 17.5 h) from acetoin and ammonium acetate \(^43\). The production of pyrazines from a culture of *Bacillus subtilis* and *B. natto* was also reported \(^44\). Therefore, it is thought that it is very likely that *E. faecalis* abundantly produces a precursor for pyrazine generation.

**Table 1** Chemical compounds in the volatile oils from MBI and MAI.

| No. | RI* | Compounds                  | Peak area (%)b | Identification methodc |
|-----|-----|----------------------------|----------------|------------------------|
|     | HP-5MS | DB-WAX |                        | MBI | MAI |
| 1   | 824    | 1263   | 2-methylpyrazine       | –   | 2.2  | RI, MS |
| 2   | 834    | 1452   | furfural               | 0.9 | –    | RI, MS |
| 3   | 864    | 1575   | 2-furanmethanol        | –   | 1.8  | RI, MS |
| 4   | 915    | 1316   | 2,5-dimethylpyrazine   | –   | 19.3 | RI, MS |
| 5   | 953    | 1722   | 5-methyl-2-furanmethanol | – | 0.9  | RI, MS |
| 6   | 959    | 1515   | phenylacetaldehyde     | 2.1 | 1.5  | RI, MS |
| 7   | 999    | 1399   | 2-ethyl-5-methylpyrazine | – | 5.4  | RI, MS |
| 8   | 1045   | 1870   | phenyl alcohol         | –   | 0.9  | RI, MS |
| 9   | 1052   | 1562   | phenylacetaldehyde     | 15.3 | 19.3 | RI, MS |
| 10  | 1068   | -      | 2,2-dihydroxy-phenylethanone | – | 0.8  | RI, MS |
| 11  | 1078   | -      | 3-ethyl-2,5-dimethylpyrazine | – | 0.6  | RI, MS |
| 12  | 1092   | -      | unknown                | –   | 0.7  | RI, MS |
| 13  | 1101   | 1392   | nonanal                | 1.7 | –    | RI, MS |
| 14  | 1116   | 1803   | phenylethyl alcohol    | –   | 9.3  | RI, MS |
| 15  | 1121   | -      | unknown                | –   | 0.4  | RI, MS |
| 16  | 1136   | 1602   | phenylacetonitrile     | –   | 0.3  | RI, MS |
| 17  | 1167   | -      | 1-phenyl-1,2-propanedione | – | 0.5  | RI, MS |
| 18  | 1192   | 2340   | chavicol               | –   | 0.6  | RI, MS |
| 19  | 1201   | 1482   | decanal                | 2.3 | –    | RI, MS |
| 20  | 1214   | -      | 3-phenylfuran          | –   | 0.5  | RI, MS |
| 21  | 1269   | 1922   | 2-phenyl-2-butenal     | –   | 1.1  | RI, MS |
| 22  | 1303   | 2277   | indole                 | 1.3 | 0.7  | RI, MS |
| 23  | 1339   | -      | indolizine             | 2.2 | –    | RI, MS |
| 24  | 1352   | -      | 6-ethyl-2-methyloctane | –   | 0.5  | RI, MS |
| 25  | 1369   | -      | 2-ethyl-3-hydroxyhexyl 2-methylpropionate | – | 0.6  | RI, MS |
| 26  | 1374   | -      | unknown                | 1.4 | –    | RI, MS |
| 27  | 1436   | -      | unknown                | –   | 0.9  | RI, MS |
| 28  | 1463   | -      | 2,6-di-tert-butylbenzoquinone | – | 0.3  | RI, MS |
| 29  | 1467   | -      | 2,4-bis-(1,1-dimethyl-1,3-phenol) | – | 0.9  | RI, MS |
| 30  | 1490   | -      | pentadecene            | –   | 0.3  | RI, MS |
| 31  | 1500   | 1500   | pentadecane            | –   | 0.6  | RI, MS |
| 32  | 1507   | 2321   | 2,4-di-tetraldehyde    | –   | 7.2  | RI, MS |
| 33  | 1561   | -      | 3,3-dimethyl-1-phenyl-1-triazine | – | 0.2  | RI, MS |
| 34  | 1647   | -      | cyclohexadecane        | –   | 0.5  | RI, MS |
| 35  | 1671   | 1755   | heptadecene            | –   | 0.3  | RI, MS |
| 36  | 1674   | -      | unknown                | –   | 0.7  | RI, MS |
| 37  | 1676   | -      | 2,2',5,5'-tetramethyl-biphenyl | – | 0.9  | RI, MS |
| 38  | 1678   | -      | diethylhexyl adipate   | 1.5 | –    | RI, MS |

* RI: Retention indices on HP-5MS and DB-WAX
b Peak area (%) was related to total detected compounds by GC-MS
c Identification method: RI, retention indice; MS, mass spectrum
However, we cannot say if there is not at all the possibility that bacteria participate, and pyrazine is formed enzymatically until the final stage. Further examination of this process is necessary, including other generation courses.

3.2 GC-O, AEDA, and OAV

The aroma-active compounds in the two oils from *E. faecalis* (MBI and MAI) were assessed, using GC-O and AEDA. Odor descriptions of the compounds detected with GC-O and the ranges of FD factors were determined. In both oils, thirteen compounds were identified; five alde-

### Table 1 Continued.

| No. | RI<sup>a</sup> HP-5MS | RI<sup>a</sup> DB-WAX | Compositions | Peak area (%)<sup>b</sup> MBI | Peak area (%)<sup>b</sup> MAI | Identification method<sup>c</sup> |
|-----|---------------------|---------------------|--------------|-----------------|-----------------|-------------------|
| 39  | 1700                | 1700                | heptadecane  | -               | 0.8              | RI, MS            |
| 40  | 1708               | -                   | 2,2-dimethyl-tetradecane | -               | 0.2              | RI, MS            |
| 41  | 1736               | -                   | unknown  | 3.8             | -               |                   |
| 42  | 1744               | -                   | 2,3-dimethyl-heptadecane | -               | 0.3              | RI, MS            |
| 43  | 1745               | -                   | *N,N*-dimethyl-4-(1H-pyrrol-1-yl)-benzenamine | 4.3             | -               | RI, MS            |
| 44  | 1762               | 1754                | 3-methyl-heptadecane | 3.6             | 0.5              | RI, MS            |
| 45  | 1788               | 2147                | (Z)-9-hexadecenal | -               | 0.4              | RI, MS            |
| 46  | 1800               | 1800                | octadecane  | 0.8             | 0.6              | RI, MS            |
| 47  | 1808               | -                   | unknown  | -               | 0.5              | RI, MS            |
| 48  | 1847               | -                   | unknown  | -               | 0.5              | RI, MS            |
| 49  | 1852               | -                   | nonadecene | 2.1             | 0.6              | RI, MS            |
| 50  | 1871               | -                   | 1-(1H-Inden-3-yl)-pyrrolidine | 1.4             | -               | RI, MS            |
| 51  | 1900               | 1900                | nonadecane | -               | 0.4              | RI, MS            |
| 52  | 1906               | -                   | 2,2-dimethyl-octane | 1.4             | -               | RI, MS            |
| 53  | 1926               | -                   | unknown  | -               | 1.6              |                   |
| 54  | 1947               | -                   | unknown  | -               | 0.3              | RI, MS            |
| 55  | 1962               | -                   | unknown  | 3.0             | -               |                   |
| 56  | 1962               | -                   | eicosene  | -               | 0.5              | RI, MS            |
| 57  | 1985               | -                   | chalcone  | -               | 0.5              | RI, MS            |
| 58  | 1991               | 2299                | (Z)-9-octadecenal  | -               | 3.3              | RI, MS            |
| 59  | 2000               | 2000                | eicosane  | 8.2             | 1.5              | RI, MS            |
| 60  | 2028               | -                   | phytan  | -               | 0.4              | RI, MS            |
| 61  | 2051               | -                   | cycloeicosane  | 1.8             | 0.4              | RI, MS            |
| 62  | 2057               | -                   | (E)-5-eicosene  | 1.6             | 0.3              | RI, MS            |
| 63  | 2099               | 2406                | (Z)-13-octadecenal  | 3.4             | 3.1              | RI, MS            |
| 64  | 2106               | 2456                | 2-hexyl-1-decanol  | 1.6             | -               | RI, MS            |
| 65  | 2107               | -                   | 5-methyl-heneicosane | -               | 0.4              | RI, MS            |
| 66  | 2148               | -                   | 1-(ethenlyoxy)-octadecane  | 1.5             | -               | RI, MS            |
| 67  | 2162               | -                   | 9-octyl-eicosane  | 1.6             | -               | RI, MS            |
| 68  | 2168               | -                   | nonadecanamide  | 2.7             | -               | RI, MS            |
| 69  | 2200               | 2200                | docosane  | 1.1             | 0.4              | RI, MS            |
| 70  | 2205               | -                   | unknown  | 1.8             | -               |                   |
| 71  | 2226               | -                   | 3,4-diphenyl-pyridine  | 1.5             | -               | RI, MS            |
| 72  | 2349               | 3321                | 9-octadecanamide  | 8.4             | 0.4              | RI, MS            |
| 73  | 2355               | -                   | 2-methyl-hexadecanal  | 2.7             | -               | RI, MS            |
| 74  | 2376               | -                   | 11-butyl-docosane  | 5.9             | -               | RI, MS            |
| 75  | 2822               | 2996                | squalene  | 7.2             | 1.5              | RI, MS            |
|     |                    |                     | total       | 100.0           | 100.0            |                   |

<sup>a</sup> RI: Retention indices on HP-5MS and DB-WAX
<sup>b</sup> Peak area (%) was related to total detected compounds by GC-MS
<sup>c</sup> Identification method: RI, retention indice; MS, mass spectrum
hydes, four alcohols, and four N-containing compounds (Table 3). All of the aroma-active components were satisfactorily identified based on their RI values and their mass spectra. The most strongly aroma-active compound, phenylacetaldehyde (peak 9; sweet, floral, FD = 64) played an important role in the characteristic aroma of MBI oil. The following aroma-active components had FD factors greater than 16: nonanal (peak 13; fat, green) and decanal (peak 19; fat). The most strongly aroma-active component of MAI oil was also phenylacetaldehyde (peak 9; sweet, floral, FD = 64). The minor characteristic odor components of MAI oil were pyrazines: 2-methylpyrrazine (peak 1; roast, FD = 1), 2,5-dimethyl-pyrrazine (peak 4; burnt, sweet, FD = 4), and 2-ethyl-5-methylpyrrazine (peak 7; burnt, sweet, FD = 8). The sniffing test and AEDA revealed both the aroma-active and aroma-characteristic components. Higher FD factors are often related to the aroma’s top note because AEDA is based on the determination of the odor threshold values of the volatile compounds. To determine the relative contribution of each of the components to the MBI and MAI odors, the OAV method was used. The OAV was obtained from the concentration and odor threshold of each component. The OAV of the aroma-active components in the oils are shown in Table 3. Because of the unavailability of odor threshold data in the literature, the OAV of 5-methyl-2-furanmethanol (peak 5) could not be determined. In the MBI oil, phenylacetaldehyde (peak 9) had the highest OAV (13388), followed by nonanal (peak 13, 5950) and decanal (peak 19, 4025), whereas in the MAI oil, phenylacetaldehyde (peak 9) had the highest OAV (18335), followed by 2-furanmethan (peak 3, 1292) and 2-ethyl-5-methylpyrrazine (peak 7, 205). These components showed particularly high FD factors, indicating that they make major contributions to the odor of the oils. In general, components with high FD factors also had high OAV, confirming the positive relationship between the FD-factor and the OAV. These components strongly participate in the odor of the oils, and the high FD factors of these components are probably a consequence of their high concentrations in the oils. The sniffing test of the original volatile oil by GC-O effectively

Table 3  Aroma-active compounds in volatile oils from MBI and MAI.

| No. | RI | Compounds | Odor description | FD-factor | OT (ppb) | concentration (ppb) | OAV |
|-----|----|-----------|-----------------|-----------|----------|---------------------|-----|
| 1   | 824 | 2-methylpyrrazine | roast | — | 1 | 60 | — | 8360 | — | 139 |
| 2   | 834 | furfural | sweet | 8 | — | 1 | 3150 | — | 3150 | — | 22 |
| 3   | 864 | 2-furanmethanol | bread | 8 | 8 | 3150 | — | 3150 | — | 2260 |
| 4   | 915 | 2,5-dimethylpyrrazine | burnt, sweet | — | 4 | 1746 | — | 73340 | — | 42 |
| 5   | 953 | 5-methyl-2-furanmethanol | burnt, sweet | — | 16 | N/A | — | 3420 | — | 22 |
| 6   | 959 | phenylaldehyde | almond | 4 | 2 | 41.7 | 7350 | 5700 | 176 | 137 |
| 7   | 999 | 2-ethyl-5-methylpyrrazine | burnt, sweet | — | 8 | 100 | — | 20520 | — | 205 |
| 8   | 1045 | phenyl alcohol | sweet | 8 | 2 | 620 | — | 3420 | — | 6 |
| 9   | 1052 | phenylacetaldehyde | sweet, floral | 1 | 32 | 64 | 53550 | 33340 | 13388 | 18335 |
| 10  | 1101 | nonanal | fat, green | 16 | — | 1 | 5950 | — | 5950 | — | 22 |
| 11  | 1116 | phenylethyl alcohol | honey, sweet | — | 16 | 1000 | — | 35340 | — | 35 |
| 12  | 1201 | decanal | fat | 16 | — | 2 | 8050 | — | 4025 | — | 22 |
| 13  | 1303 | indole | burnt | 2 | 2 | 140 | 4550 | 2660 | 33 | 19 |

**Notes:**
- a: Retention index on HP-5MS.
- b: Compounds are listed in order of their elution time from a HP-5MS column.
- c: FD-factor on HP-5MS column.
- d: The sample concentration (mg/mL) was assigned on FD-factor of 1.
- e: Odor threshold measured in water solution and represented as mg of compound / kg of water (ppb).
- f: The OAV was obtained by dividing the concentrations of the odors by their thresholds.
- g: Data not available.
- ND: Not determined.
determined key aroma compounds. In summary, taking the FD factor and OAV into account, we determined that phenylacetaldehyde (peak 9, sweet, floral) has an important role in the aroma of both oils. In addition, nonanal (peak 13, fat, green), and decanal (peak 19; fat) also contribute to the aroma of the MBI oil (Fig. 1). On the other hands, 5-methyl-2-furanmethanol (peak 7; burnt, sweet) and phenylethyl alcohol (peak 14; honey, sweet) are important components in the MAI oil (Fig. 1). These components in the MAI oil have a sweet-floral-burnt like odor, as determined by the sniffing test. The characteristic odorants identified to contribute to this odor of the MAI oil were pyrazines and alcohols produced by E. faecalis.

4 CONCLUSIONS

To the best of our knowledge, this is the first study to indicated the biodiversity of the industrial cultivation medium after incubation with E. faecalis and identify the key aroma-active components responsible for the overall aroma of the MAI. The chemical compositions of both the MBI and MAI oils were described in detail. It is notable that the MAI afforded various aroma-active pyrazines and alcohols not found in the MBI. Further studies are needed to determine the biosynthetic pathway by which each component, detected exclusively in the MAI, is produced during fermentation. We expect that these results will be useful for future investigations into the utilization of liquid medium from fermentation processes.

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