Short Communication

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Comprehensive two-dimensional gas chromatographic analysis of volatile organic compounds in distillate of fermented Sorbus domestica fruit

Abstract: The Slovak-Czech spirit distilled from Sorbus domestica was analyzed by GCxGC with headspace solid phase microextraction and liquid-liquid extraction. Two column combinations, nonpolar × medium-polar and polar × medium-polar were tested. The second column setup provided more exploited 2D separation space with better distribution of peaks in chromatogram. Effects of the first column on the elution order of γ-lactones, benzyl esters, and unsaturated aldehydes in the second separation were observed. More than 500 compounds were detected; nearly 100 were confirmed with standards.

Keywords: Sorbus domestica, Oskorošovica, two-dimensional gas chromatography, alcoholic beverage

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1 Introduction

According to the latest WHO report [1] the annual consumption of alcohol in EU is 12.5 L of pure alcohol per capita, twice the world average. The popularity of various alcoholic beverages is nearly constant: 34.6% spirits, 37.1% beer, 26.4% wine and 2.5% others [2]. A combination of low price and legislated quality promotes alcohol production in central-eastern and eastern European countries. In this region a variety of fruit spirits are traditional. Although common fruit such as plums, apples, pears, or cherries is usually employed, unconventional fruit such as those of the service tree (Sorbus domestica) are also fermented.

Service trees grow in western, central and southern Europe, northwest Africa and southwest Asia [3,4], although they are very rare and threatened in many European countries [5]. The 2-3 cm greenish to brownish apple or pear shaped fruit have been greatly valued since Roman times [6].

The phenolic content of Sorbus domestica fruit at different stages of maturity and well matured fruit pulp was determined to compare their antioxidant properties [7-9]. It is rich in flavonoids, mostly quererin, hydroxybenzoic acids, hydrocinnamic acids and their derivatives. This suggests a high aldose reductase inhibitory activity, having antidiabetic properties [10]. Ethyl carbamates in distilled beverages have been determined [11].

Sorbus domestica (Oskorušovica) fruit distillates are often valued by experts due to their specific aroma and taste characteristics, which are defined by presence of particular volatile organic compounds (VOCs). In general, the composition of an alcoholic beverage is determined by raw material, flavor additives, and processing stages such as fermentation, distillation and maturation [12,13]. Raw material is a main source of terpenes and their derivatives, which are responsible for primary aroma and taste of beverage products. Since the majority of terpenes is semi-volatile and linked to sugar molecules, fruit ripen process and conditions of hydrolysis are responsible for the overall aroma perception of alcoholic spirit. Other important compounds that determine overall taste of alcoholic beverages are their major constituents such as alcohols, carboxylic acids and their esters, carbonyl compounds etc. Those are however formed during the fermentation of sugar, thus determine the secondary taste and aroma perception of alcoholic beverage.

Gas chromatography is especially suited to determining volatile organic compounds (VOCs) in
food and beverages. Comprehensive two-dimensional gas chromatography (GC×GC), based on the theory of multidimensional separation [12], has enhanced separation and resolution power. The heart of GC×GC is the combination of two columns with different selectivity connected in series through a special interface called a modulator [13-15]. Ideally, stationary phase selection demands orthogonality - independent separation mechanisms in the two columns. Giddings [16] defined order and disorder characteristics of multidimensional separations. Orthogonality and capacity have been described based on fractal analysis [17], information theory and a geometric method [18]. There have been efforts to predict 2nd dimension retention based on one-dimensional GC [19,20]. The necessity of truly orthogonal separation is an open question for practical analysis.

The aim of this work was to determine the VOC profile of distillates from Sorbus domestica fruit using GC×GC-TOFMS.

2 Experimental procedure

2.1 Chemicals

The three samples were obtained from Rudolf Jelinek distillery (Czech Republic), Bošáca distillery, and Bird Valley distillery (both in Slovakia). Anhydrous NaCl was supplied by Microchem (Pezinok, Slovakia), and the C7-C30 alkane mixture used to calculate retention indices was purchased from Supelco (Bellefonte, PA, USA). Solution of benzophenone in ethanol with concentration 1.6 mg L⁻¹ was used as an internal standard solution.

2.2 Sample preparation

2.2.1 Liquid-liquid extraction (LLE)

A mixture of 30 mL sample with 2 g NaCl was extracted with three 10 mL portions of pentane. The combined extracts were concentrated to 1.5 mL in a Kuderna-Danish apparatus at 43°C. One microliter of concentrate was injected into the GC in splitless mode.

2.2.2 Solid phase microextraction (SPME)

A 5 mL aliquot of distillate with 0.5 g dissolved NaCl was transferred to a 20 mL capped glass vial. The vial was heated at 40°C and stirred at 400 rpm for 30 min to equilibrate liquid and vapor, then the organic vapors were adsorbed on a SPME fiber coated with 50/30 mm DVB/ CAR/PDMS for 30 min at 40°C. The sample was desorbed at 220°C for 2 min in the GC injector in splitless mode.

2.3 Gas chromatography-mass spectrometry analysis

Analysis was performed using a Pegasus 4D GC×GC-TOFMS instrument (LECO, St. Joseph, MI, USA), including an Agilent 6890 GC (Agilent Technologies, Palo Alto, CA, USA), time of flight mass spectrometer (LECO) and Gerstel MPS2 autosampler (Gerstel, Mülheim, Germany). A nonpolar × medium-polar column combination consisting of HP-PONA 45 m × 0.2 mm × 0.5 μm (Agilent) in the first dimension and BPX-50 1.5 m × 0.1 mm × 0.1 μm film (50 % diphenyl - 50% dimethyl polyphenylene-siloxane; SGE Analytical Science, Victoria, Australia) in the second dimension was used. The reversed column setup tested polar × medium-polar DB-FFAP 30 m × 0.25 mm × 0.25 μm film (polyethylene glycol modified by nitroterephthalic acid, Agilent) in the first and BPX-50 in the second dimension. The helium carrier flow was 1 mL min⁻¹. An initial temperature of 40°C held for 10 min, followed by a gradient at 2°C min⁻¹ to 240°C, then held for 10 min was used in all analyses. A dual stage jet cryogenic (liquid N₂) modulator was operated at 30°C higher temperature than actual oven temperature, with a 6 s modulation period. The second column was operated at 10°C higher than the primary column. The transfer line was at 240°C.

The TOFMS ion source of was at 230°C and the detector voltage was 1700 V. Electron ionization energy was 70 eV. The signal from m/z 29-300 was acquired at 100 spectra/s. The chromatograms were evaluated using LECO ChromaTOF 4.21 software and the US National Institute of Standards and Technology (NIST08) mass library.

3 Results and discussion

3.1 Sample preparation

Sample preparation is crucial for complicated matrices with a wide concentration range like alcoholic beverages. A range of procedures have been suggested for VOC
determination in alcoholic beverages [21-23]; the most popular were headspace solid-phase (HS-SPME) and classical liquid-liquid extraction (LLE). We compared these for the analysis of spirit distilled from Sorbus domestica.

The optimization procedure of both, LLE and HS-SPME conditions for extraction of VOC from fruit spirits was described in our previous work [29].

Commercially available fiber with a triple phase DVB/CAR/PDMS coating was considered most suitable for wine [24-26]. However, we found that HS-SPME sorption was inferior to pentane extraction as terpenes (farnesol isomers, 4-terpinenol, cis-carveol), phenolic compounds (acetophenone, 1-phenylethanol, vanillin), ethyl esters of some carboxylic acids, and alcohols with functional groups at C2 and C3 positions (2-hexanol, 3-hexanol, 2-hexenal, 3-methyl-2-buten-1-ol, 2-hexen-1-ol) were not concentrated by HS-SPME. On the other hand, LLE into pentane was not completely effective for aliphatic alkanes; alcohols with OH at C1, methyl carboxylates, benzaldehyde, and 5-methylfuran were partially excluded. The significant area (%) differences between results obtained with HS-SPME and LLE were observed for 1-butanol (1.39 and 0.01%), 3-methyl-1-butanol (10.10 and 0.64%), 3-pentanol (0.01 and 1.11%), 1-hexanol (3.25 and 0.23%) and limonene (1.75 and 0.09%). Because a broader range of VOCs was concentrated by LLE, this procedure was used. The repeatability of SPME and LLE sample treatment procedures were calculated for 25 randomly selected compounds belonging to various classes such as organic acids and their esters, alcohols, terpenes or furan derivatives, and are present on chromatogram at different concentration levels. As was expected, the most found RSD values vary within range 0.2-3.5 independently on used sample treatment procedure. The low RSD values are related to use of internal standard method. The highest RSD value (4.8%) was observed for acetic acid extracted using SPME.

### 3.2 The column combinations

Although effective selection of column combination in comprehensive two-dimensional gas chromatography is determined separation orthogonality, sufficient results have been also obtained in the case of application of non-orthogonal approach [27]. Both conventional (nonpolar × medium-polar) and “reversed” (polar × medium-polar) column setups were studied. Either a HP-PONA or DB-FFAP column was the first dimension, and BPX-50 was the second. The GC×GC-TOFMS chromatograms are shown on Fig. 1. The retention times and retention indices are in Table 1.

The reversed elution order of γ-lactones, benzoic acid esters and unsaturated aldehydes in the second dimension is significant; the first dimension separation may affect the second dimension retention time. This possibility was mathematically described by Phillips et al. [28]. The short separation time on the 2nd column means that it is carried out isothermally and the dominant separation mechanism is thermodynamically controlled. An elution order change for a mixture of phenols and aromatic compounds has been explained by thermodynamic parameters [29].

In the case of “reversed” combination of columns, 2D separation space is more broadly exploited with a better peak distribution (Fig. 2). In addition this column set shows an ordered chromatographic profile for the most common classes of compounds in alcoholic beverages, which simplifies the evaluation of results. Even though the alkanes were separated and identified only using the HP-PONA column in the first dimension, the “reversed” set was more suitable for the studied beverages. In addition, other alcoholic beverages [30], and wine [31,32] have been successfully analyzed using polar × medium-polar columns.

### 3.3 VOC profile characterization

More than 500 chromatographic peaks were detected by GC×GC-TOFMS, and nearly 100 have been confirmed with standards (Table 1). All compounds shown in Table 1 were found in all studied samples regardless on producer. In order to express relative content of compounds, the area % was determined based on average peak areas calculated from peaks areas obtained for the same compound in all studied samples. The RSD values of peak areas between samples vary within a range from 3 to 9%. The highest RSD value (9.8%) was observed estragol using SPME as sample treatment method. On the contrary, the lowest RSD value was observed for hexanol. As can be seen from the results in Table 1, the majority of compounds has been also identified in other alcoholic beverages, e.g. fruit distillate, brandy, cognac, rum, and whisky.

Aliphatic (C₃-C₁₈) carboxylic acid esters (methyl and ethyl) were the dominant compounds found (Fig. 3), and this tendency was also observed for liquor [36], cognac [38], whisky [45], orange spirit [44], and grape marc spirit [47]. However, another result was reported for aged rum [52], where alcohols were a main class. In general, methyl
| No | Compound                                   | Ref.          | Area (%) | Ref. | DB-FFAP RT (min) | DB-FFAP RT (s) | PONA RT (min) | PONA RT (s) |
|----|-------------------------------------------|---------------|----------|------|-----------------|----------------|---------------|-------------|
| 1  | 2-Butanol*                                | [35-37]       | 0.03     | 0.90 | 11.80           | 1.44           | <             | 10.20       |
| 2  | Butanoic acid, ethyl ester*              | [35, 38-43]   | 1.11     | 2.58 | 12.10           | 2.63           | <             | 25.40       |
| 3  | 1-Propanol*                               | [35, 38, 40, 44] | 0.59    | 1.40 | 12.90           | 1.37           | <             |             |
| 4  | 2-Methylbutanoic acid, ethyl ester*      |               | 0.01     | 0.01 | 13.00           | 2.98           | 1007          |            |
| 5  | Dimethyl disulfide*                       | [37]          | 0.06     | 0.14 | 14.00           | 2.27           | 1036          |             |
| 6  | 2-Hexanone*                               | [37]          | 0.02     | 1.43 | 15.80           | 4.06           | 1185          |             |
| 7  | 2-Methyl-1-propanol*                     | [35, 40-44]   | 0.50     | 1.40 | 16.70           | 1.63           | 1089          |             |
| 8  | 3-Pentanol*                               | [37]          | 0.01     | 1.11 | 17.50           | 1.61           | 1108          |             |
| 9  | 2-Pentanol*                               |               | 0.05     | 0.66 | 18.30           | 1.61           | 1124          |             |
| 10 | 4-Methyl-3-penten-2-one*                 |               | 0.05     | 0.75 | 19.10           | 1.48           | 1132          |             |
| 11 | Pentanoic acid, ethyl ester*             | [36-38]       | 0.02     | 0.10 | 19.30           | 1.48           | 1124          |             |
| 12 | 1-Butanol*                                | [35-40]       | 1.39     | 0.01 | 19.80           | 3.14           | 1124          |             |
| 13 | 2-Heptanone*                             | [39, 41]      | 0.06     | 0.21 | 21.80           | 2.75           | 1174          |             |
| 14 | Heptanoin*                                | [39, 43]      | 0.07     | 0.08 | 22.00           | 2.75           | 1179          |             |
| 15 | Hexanoic acid, methyl ester*             | [36, 37, 39]  | 0.09     | 0.12 | 22.20           | 2.88           | 1182          |             |
| 16 | Limonene*                                | [36, 39, 44, 45] | 1.75   | 0.09 | 22.40           | 4.06           | 1185          |             |
| 17 | 3-Hexanol*                               | [47]          | 0.30     | 1.20 | 23.50           | 1.63           | 1194          |             |
| 18 | 3-Methyl-1-butanol*                      | [35-37, 39]   | 10.10    | 0.64 | 23.50           | 1.61           | 1210          |             |
| 19 | Dodecane*                                |               | 0.02     | 0.00 | 24.50           | 0.90           | 1204          |             |
| 20 | 2-Hexenal*                               | [48]          | 0.03     | 2.40 | 24.40           | 2.34           | 1217          |             |
| 21 | 2-Hexanol*                               | [37]          | 0.29     | 0.17 | 24.50           | 1.7            | 1218          |             |
| 22 | 2-Pentylfuran*                           | [38]          | 0.33     | 0.09 | 25.30           | 3.07           | 1228          |             |
| 23 | Hexanoic acid, ethyl ester*              | [35, 36, 38, 40, 44, 45] | 7.51   | 3.21 | 25.30           | 3.29           | 1231          |             |
| 24 | p-Cymene*                                | [41, 44, 46]  | 0.43     | 0.04 | 27.60           | 3.37           | 1261          |             |
| 25 | 2-Octane*                                | [37]          | 0.04     | 0.07 | 28.90           | 2.89           | 1280          |             |
| 26 | 2-Heptanol*                              | [38-40]       | 0.15     | 0.85 | 31.50           | 1.79           | 1316          |             |
| 27 | 3-Methyl-2-buten-1-ol*                   | [35, 37]      | 0.08     | 0.04 | 31.50           | 1.48           | 1318          |             |
| 28 | Heptanoic acid, ethyl ester*             | [41, 45]      | 0.55     | 0.26 | 32.40           | 3.42           | 1331          |             |
| 29 | Propanoic acid, 2-hydroxy-, ethyl ester* | [35, 36, 40]  | 0.35     | 0.14 | 33.10           | 1.55           | 1345          |             |
| 30 | cis-Rose oxide*                          | [46, 49]      | 0.04     | 0.04 | 33.30           | 3.51           | 1346          |             |
| 31 | 1-Hexanol*                               | [35, 38, 41, 44, 45] | 3.25   | 0.23 | 33.70           | 1.68           | 1352          |             |
| 32 | trans-Rose oxide*                        | [46]          | 0.01     | 0.01 | 34.20           | 3.71           | 1358          |             |
| 33 | cis-3-Hexen-1-ol*                        | [35, 41]      | 0.02     | 0.09 | 35.80           | 1.65           | 1381          |             |
| No | Compound                                      | Ref.                   | Area (%)* | HS-SPME | LLE | DB-FFAP | PONA |
|----|-----------------------------------------------|------------------------|-----------|---------|------|---------|------|
| 34 | Fenchone*                                     | [50]                   | 0.01      | 0.01    | 35.90| 3.24    | 1384 |
| 35 | Octanoic acid, methyl ester*                 | [39, 40, 44, 45]       | 1.13      | 0.35    | 36.20| 3.17    | 1387 |
| 36 | Nonanal*                                      | [39-42, 44]            | 0.07      | 0.30    | 36.40| 3.24    | 1391 |
| 37 | 3-Octanol*                                    | [35, 38-40]            | 0.13      | 0.37    | 36.50| 1.98    | 1391 |
| 38 | 2-Hexen-1-ol*                                | [51]                   | 0.01      | 0.01    | 37.20| 1.65    | 1403 |
| 39 | Thujone*                                      | [52]                   | 0.14      | 0.46    | 43.50| 0.83    | 1502 |
| 40 | 1-Octen-3-ol*                                | [36, 37, 39]           | 0.22      | 0.77    | 40.10| 1.74    | 1448 |
| 41 | Acetic acid*                                  | [38, 40, 44, 45]       | 0.06      | 0.15    | 40.70| 1.34    | 1458 |
| 42 | Linalool oxide isomer I*                     | [38, 39, 44, 46]       | 0.33      | 0.29    | 39.30| 2.30    | 1434 |
| 43 | Octanoic acid, ethyl ester*                  | [35, 38-40, 44, 45]    | 0.01      | 1.25    | 39.40| 3.77    | 1434 |
| 44 | Linalool oxide isomer II*                    | [35, 39, 44, 46]       | 0.28      | 0.09    | 42.50| 1.81    | 1488 |
| 45 | Furfural*                                     | [35, 38, 44, 45]       | 0.67      | 0.93    | 41.30| 1.59    | 1467 |
| 46 | 2-Ethyl-1-hexanol*                            | [37]                   | 0.03      | 0.09    | 42.50| 1.81    | 1488 |
| 47 | Nonanoic acid, methyl ester*                 | [38, 40, 44, 45]       | 0.28      | 0.09    | 42.70| 3.29    | 1489 |
| 48 | Pentadecane*                                  | [39, 44]               | 0.18      | 0.15    | 43.50| 0.83    | 1502 |
| 49 | 2-Nonanol*                                    | [41]                   | 0.12      | 0.06    | 44.40| 2.04    | 1517 |
| 50 | Benzaldehyde*                                 | [35, 38-41, 44, 46]    | 3.21      | 0.01    | 44.60| 2.14    | 1520 |
| 51 | Nonanoic acid, ethyl ester*                  | [38, 39, 44-46]        | 0.09      | 0.82    | 45.50| 3.92    | 1533 |
| 52 | Linalool*                                     | [38, 39, 44, 46]       | 0.07      | 0.12    | 46.10| 1.97    | 1545 |
| 53 | 5-Methylfurfural*                             | [38, 40]               | 0.02      | 0.02    | 47.80| 1.85    | 1573 |
| 54 | Decanoic acid, methyl ester*                 | [38, 39, 43, 45]       | 0.41      | 0.51    | 48.80| 3.46    | 1590 |
| 55 | 4-Terpinenol*                                | [38, 39]               | 0.04      | 0.04    | 49.00| 2.46    | 1593 |
| 56 | Hexadecane*                                   | [39, 44]               | 0.02      | 0.02    | 49.50| 0.79    | 1602 |
| 57 | Decanoic acid, ethyl ester*                  | [35, 38-41, 44, 45]    | 0.01      | 0.03    | 51.80| 4.44    | 1643 |
| 58 | Acetophenone*                                 | [38, 40]               | 0.02      | 0.02    | 52.00| 2.09    | 1646 |
| 59 | Estragole*                                    | [38, 40]               | 0.07      | 0.01    | 53.10| 2.48    | 1666 |
| 60 | 4-Oxoisophorone*                              | [38]                   | 0.01      | 0.01    | 54.20| 2.23    | 1686 |
| 61 | Borneol*                                      | [38]                   | 0.13      | 0.47    | 54.50| 2.05    | 1691 |
| 62 | α-Terpineol*                                  | [38, 40, 46]           | 0.03      | 0.11    | 54.50| 2.16    | 1691 |
| 63 | Camphor*                                      | [38]                   | 0.14      | 0.30    | 54.60| 2.04    | 1500 |
| 64 | 2-Nonen-1-ol*                                 | [38]                   | 0.12      | 0.03    | 55.70| 1.88    | 1713 |
| 65 | Epoxyxylinalol isomer I*                     | [38]                   | 0.01      | 0.12    | 56.80| 1.89    | 1734 |

Continued Table 1: Compounds identified in spirit distilled from *Sorbus domestica* by GCxGC-TOFMS.
Continued Table 1: Compounds identified in spirit distilled from *Sorbus domestica* by GCxGC-TOFMS.

| No | Compound                              | Ref.                  | Area (%)* | HS-SPME | LLE | RT 1D | RT 2D | RI 1D | RI 2D | RI | DB-FFAP | PONA |
|----|---------------------------------------|-----------------------|-----------|---------|------|-------|-------|-------|-------|----|---------|-------|
|    |                                       |                       |           |         |      |       |       |       |       |    |         |       |
| 67 | Undecanoic acid, ethyl ester*         | [40, 41, 43]          | 0.52      | 0.08    | 57.00| 3.85  | 1738  | 78.30 | 1.73  | 1469 | 1.73   |       |
| 68 | Epoxylinalol isomer II*               | [40, 41]              | 0.05      |         | 58.20|       | 1.91  |       |       |     |         |       |
| 69 | Citronellol*                          | [38, 44]              | 0.02      | 0.09    | 58.40| 1.92  | 1764  | 60.70 | 1.83  | 1200 | 1.83   |       |
| 70 | Dodecanoic acid, methyl ester*        | [40, 41]              | 0.42      | 0.22    | 60.20| 3.63  | 1798  | 80.00 | 1.76  | 1497 | 1.76   |       |
| 71 | 1-Phenylethanol*                      | [52]                  | 0.01      |         | 60.90| 1.61  | 1812  |       |       |     |         |       |
| 72 | cis-Carveol*                          | [46]                  | 0.01      |         | 61.90| 1.91  | 1831  |       |       |     |         |       |
| 73 | Dodecanoic acid, ethyl ester*         | [35, 38, 39, 41]      | 0.50      | 0.47    | 62.50| 4.23  | 1843  | 80.0  | 1.76  | 1497 | 1.76   |       |
| 74 | Geraniol*                             | [35]                  | 0.06      |         | 62.70| 1.88  | 1847  | 62.50 | 1.89  | 1226 | 1.89   |       |
| 75 | Nonadecane*                           | [53]                  | 0.01      |         | 65.40| 0.89  | 1900  | 101.1 | 1.62  | 1890 | 1.62   |       |
| 76 | Tridecanoic acid, ethyl ester*        |                       | 0.03      | 0.04    | 67.50| 4.02  | 1943  | 89.70 | 1.75  | 1667 | 1.75   |       |
| 77 | Tetradecanoic acid, methyl ester*     | [37]                  | 0.03      | 0.07    | 70.50| 3.81  | 2004  | 91.30 | 1.79  | 1696 | 1.79   |       |
| 78 | Phenol*                               | [36]                  | 0.01      |         | 70.70| 1.31  | 2009  |       |       |     |         |       |
| 79 | Nerolidol isomer II*                  | [38, 40, 41]          | 0.02      | 0.09    | 72.10| 2.49  | 2038  | 82.6  | 1.84  | 1541 | 1.84   |       |
| 80 | Heneicosane*                          |                       | 0.01      |         | 75.00| 0.86  | 2100  |       |       |     |         |       |
| 81 | Pentadecanoic acid, ethyl ester*      | [38, 40, 41]          | 0.08      |         | 77.10| 4.18  | 2148  | 99.90 | 1.79  | 1865 | 1.79   |       |
| 82 | Eugenol*                              | [36, 41, 42]          | 0.09      |         | 78.00| 1.77  | 2168  | 69.10 | 2.28  | 1323 | 2.28   |       |
| 83 | Hexadecanoic acid, ethyl ester*       | [35, 38-41, 43]       | 0.09      |         | 81.60| 4.25  | 2251  | 103.3 | 1.91  | >    |         |       |
| 84 | Farnesol isomer II*                   | [38, 41, 45]          | 0.06      |         | 86.00| 2.27  | 2356  | 91.10 | 1.99  | 1693 | 1.99   |       |
| 85 | Heptadecanoic acid, ethyl ester*      |                       | 0.03      |         | 86.00| 4.34  | 2356  |       |       |     |         |       |
| 86 | Octadecanoic acid, ethyl ester*       |                       | 0.22      |         | 90.10| 4.47  | 2458  |       |       |     |         |       |
| 87 | Vanillin*                             | [35, 40]              | 0.01      |         | 94.50| 1.61  | >     |       |       |     |         |       |
| 88 | Nonadecanoic acid, ethyl ester*       |                       | 0.01      |         | 97.90| 4.57  | >     |       |       |     |         |       |
| 89 | Heptane*                              |                       |          |         |       |       |       |       |       |     | 1.37   | 700   |
| 90 | Octane*                               |                       |          |         |       |       |       |       |       |     | 1.35   | 796   |
| 91 | Nonane*                               | [53]                  |          |         |       |       |       |       |       |     | 1.38   | 895   |
| 92 | (-)-β-Pinene*                         | [46]                  |          |         |       |       |       |       |       |     | 1.49   | 928   |
| 93 | Heptadecane*                          | [53]                  |          |         |       |       |       |       |       |     | 1.55   | 1689  |
| 94 | Farnesol isomer I*                    |                       |          |         |       |       |       |       |       |     | 2.00   | 1673  |
| 95 | Octadecane*                           | [53]                  |          |         |       |       |       |       |       |     | 1.57   | 920   |

a) Sigma-Aldrich (St. Louis, USA); b) Merck KGaA (Darmstadt, Germany); c) SAFC Biosciences (Lenexa, USA); d) Supelco (Bellefonte, PA, USA); * - odour active compounds defined by http://www.flavornet.org/flavornet.html; RT – retention time; RI – retention index; Area (%)* – the ratio of the compound peak area related to the internal standard response to total peaks areas of identified compounds obtained on DB-FFAP column, where peak area was calculated relative to the internal standard area.
Figure 1: GCxGC-TOF-MS chromatograms using columns (A) HP-PONA and BPX-50, (B) DB-FFAP and BPX-50.

Figure 2: Distribution of main compound classes in *Sorbus domestica* beverage.
and ethyl esters are formed during fermentation of fruit molasses. Among them, esters of butanoic (2.58%), hexanoic (3.21%) and octanoic (1.25%) acids are the main components present in studied distillates. Ethyl butanoate, ethyl hexanoate, and ethyl octanoate are the major components present. Other important VOCs were aliphatic fusel alcohols and the aromatic 1-phenylethanol. Aliphatic fusel alcohols and an aromatic alcohol 1-phenylethanol were found at significant concentration levels, while the highest relative content was observed for 1-propanol (1.40%), 2-methyl-1-propanol (1.40%), 2-butanol (0.91%), 3-methyl-1-butanol (0.64%), 2-pentanol (0.66%), 3-pentanol (1.11%).

Similarly, 3-methyl-1-butanol was shown as the most abundant higher alcohols in liquor [37], whisky [45], pear distillates [54] and brandies [55]. High contents of 1-propanol, 2-methyl-1-propanol, 2-butanol, 3-methyl-1-butanol, 2-pentanol, and 3-pentanol were found. In addition, the presence of the unsaturated fusel alcohols 3-methyl-2-buten-1-ol, 2-hexen-1-ol and 1-octen-3-ol are notable. The carbonyl compounds include linear and branched aldehydes (2-hexenal, heptanal, nonanal), and ketones (4-methyl-3-penten-2-one, 2-hexanone, 2-heptanone, 2-octanone). Acetic acid was the main volatile acid found; it was confirmed with the authentic standard. A variety of terpenes and their derivatives (e.g. limonene, cis- and trans-rose oxide, fenchone, thujone, linalool, epoxylinalol, citronellol, geraniol, farnesol, nerolidol) with borneol, linalool oxides and α-terpineol as major components were detected. Similarly to the other alcoholic beverages, such as brandy, gin and rum [36,46,52], linalool oxides and α-terpineol were the major components from group of terpenes. On the contrary, the fruit distillates were characterized by a higher content of borneol (0.47 %). The identification of dimethyl sulfide suggests that sulfur amino acid metabolism occurred. 5-methylfurfural and eugenol previously described as products of maturation [35] were also identified by GCxGC-TOFMS.

4 Conclusions

A complete comprehensive two-dimensional chromatographic analysis of spirit distilled from fermented *Sorbus domestica* was performed. Two sample preparation methods were first compared, HS-SPME and LLE with pentane. The latter more effectively concentrates some terpenes (farnesol isomers, 4-terpinenol, cis-carveol), phenolic compounds (acetophenone, 1-phenylethanol, vanillin), C15-C18 fatty acid ethyl esters, and alcohols with OH groups at C2 and C3 (2-hexanol, 3-hexanol, 2-hexenal, 3-methyl-2-buten-1-ol, 2-hexen-1-ol). Esters of aliphatic (C3-C18) carboxylic acids constitute a main class of VOCs in the studied fruit distillate, whereas aliphatic alcohols fraction was composed mainly by 1-propanol (1.40%), 2-methyl-1-propanol (1.4%), 2-butanol (0.91%), 3-methyl-1-butanol (0.64%), 2-pentanol (0.66%), 3-pentanol (1.11%).

Bornanol (0.47%), linalool oxides (0.29% and 0.27%) and α-terpineol (0.11%) were the most abundant terpene compounds detected in the distillate from *Sorbus domestica* fruits. A “reversed” (polar × medium-polar) design improved the separation with better peak distribution than a traditional setup (nonpolar × medium-polar). Further, the usual chromatogram order for common compound classes was retained, confirming suggestions from a non-orthogonal approach to column selection. The reversed second dimension elution order of γ-lactones, benzoic acid esters, and unsaturated aldehydes was caused by thermodynamic control of the separation mechanism on the medium-polar column. Many volatile organic compounds responsible for the taste and odor of alcoholic beverages were found, and the presence of nearly 100 compounds was confirmed by standards.

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